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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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Pulmonary Toxicity of Carmustine in Patients Treated for Malignant Glioma[1,2]

Alan S. Weinstein, Marie Diener-West, Diana F. Nelson, and E. Pakuris[3,*]

Carmustine (BCNU) was employed as the only chemotherapeutic agent in a Radiation Therapy Oncology Group multimodality study comparing misonidazole-radiosensitized radiation therapy to conventional radiation therapy in 318 patients with malignant glioma. In 289 patients evaluable for BCNU pulmonary toxicity, there were no clinical manifestations of toxicity in patients receiving < 902-mg/m^2 total BCNU dose. Ten of 107 patients receiving more than this dose developed detectable pulmonary toxicity. Results of a multivariate regression analysis of risk factors, which corrects for survival time bias, suggested increased risk of pulmonary toxicity when total dose exceeds 1400 mg/m^2. The risk of pulmonary toxicity was not increased by the administration of misonidazole and does not appear to be related to age. [Cancer Treat Rep 70:943-946, 1986]

In the last 5–6 years, several small series and multiple case reports have appeared in the literature documenting the potential for pulmonary toxicity with carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; BCNU] chemotherapy (1–4). Although pulmonary toxicity has been documented with as little as approximately 300-mg/m^2 total dose (5), most of the data accumulated in the literature point to a cumulative dose dependence of BCNU pulmonary toxicity (6).

A decrease in the diffusion capacity for carbon monoxide is one of the most sensitive indicators of early BCNU pulmonary toxicity (4). Dyspnea, tachypnea, and a dry hacking cough are the frequent clinical symptoms that develop later. On physical examination, basilar rales or rhonchi may be heard. A restrictive pattern on pulmonary function studies may be observed. When the chest x-ray becomes abnormal with interstitial infiltrates, the process may be irreversible.

This paper evaluates the incidence of pulmonary toxicity in patients with malignant glioma who received radiation therapy with and without misonidazole and were treated with BCNU as a single agent. It confirms the dose-related incidence suggested previously and better defines the risk in patients treated with BCNU in a large, multi-institutional group protocol.

MATERIALS AND METHODS

From study initiation in November 1979 to closure in January 1983, 318 patients with malignant glioma were entered onto the Radiation Therapy Oncology Group (RTOG) Protocol 79-18. In this randomized phase III trial, the effectiveness of the radiation sensitizer misonidazole was evaluated in patients with malignant glioma. Patients were treated with either radiation therapy plus BCNU or misonidazole-sensitized radiation therapy plus BCNU. The BCNU was given in both arms similarly: 80 mg/m^2 iv on Days 3, 4, and 5 of the first week of radiotherapy and repeated every 8 weeks thereafter. Initially, treatment was continued for 2 years, or until disease progression or death. However, in August 1981, after accumulating data became available in the literature suggesting dose-dependent BCNU pulmonary toxicity, a protocol addendum was distributed suggesting that the total dose be limited to 1200-1500 mg/m^2.

All patients on this study received corticosteroids during the first 7–8 weeks while receiving radiotherapy. The dose prescribed was a minimum of 3 mg of dexamethasone, or the equivalent dose in other corticosteroids, daily during the radiotherapy course. The corticosteroid was then tapered and stopped, if possible. Furthermore, approximately one-half of the patients were randomized to receive misonidazole, 2.5 g/m^2 orally, once weekly during the first 6 weeks of radiotherapy.

Beginning in October 1981, due to the increasing reports of BCNU pulmonary toxicity, a retrospective and ongoing analysis of each case was made with respect to the appearance of pulmonary toxicity. Since over one-half of the patients had been entered before the start of this analysis and no formal pulmonary function testing

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[2] Supported in part by Public Health Service grants CA-32115 and CA-21661 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3] Radiation Therapy Oncology Group, American College of Radiology, Philadelphia, PA.
*_Reprint requests to:_ Headquarters, Radiation Therapy Oncology Group, American College of Radiology, 925 Chestnut St, Philadelphia, PA 19107.

had been required for entry or follow-up on the study, only clinical signs and symptoms of pulmonary toxicity were evaluated routinely.

Pulmonary toxicity was graded from 1 to 5. *Grade 1* was defined by mild symptoms with a 20%–50% decrease in diffusing or vital capacity; *grade 2* was moderate symptoms with a > 50% decrease in diffusing or vital capacity; *grade 3* was severe symptoms requiring intermittent oxygen; *grade 4* required continuous oxygen or assisted ventilation; and *grade 5* was pulmonary toxicity resulting in death.

Statistical Methods

Among the 318 patients placed on study, 29 cases were excluded; 23 were ineligible, cancelled, or unevaluable, two had no follow-up forms, and four had no chemotherapy forms. In 289 patients evaluable for BCNU pulmonary toxicity, none was detected in 182 patients receiving < 902-mg/m^2 total BCNU dose. Ten of 107 patients receiving more than this dose developed detectable pulmonary toxicity.

A number of factors were investigated for their potential relationship to BCNU pulmonary toxicity through a proportional hazard regression analysis (7). An event was defined as the occurrence of pulmonary toxicity and the time to the event was the number of elapsed days between the start of BCNU chemotherapy and the development of the first symptoms of pulmonary toxicity. Observations were censored at time of death or time of last follow-up. This type of adjustment for differences in survival time was used in order to ascertain the relationship between receiving misonidazole, age, and BCNU dose and the incidence of pulmonary toxicity. Treatment with misonidazole and continuous age were fixed covariates, whereas BCNU dose was defined as a time-dependent covariate.

RESULTS

Among the 289 patients available for analysis, no toxicity was observed in 182 patients who received < 902-mg/m^2 total BCNU dose. In patients receiving between 902 mg/m^2 and 2062 mg/m^2, ten of 107 patients at risk developed BCNU pulmonary toxicity. In each of these ten patients, the onset of toxicity occurred after the completion of a cycle of BCNU, and BCNU chemotherapy was discontinued. The maximum pulmonary toxicity by clinical grade was generally mild to moderate, with three grade 1 and two grade 2 toxicity scores. However, one patient developed grade 3 toxicity, and there were three deaths attributed to BCNU pulmonary toxicity.

One of the cases of fatal (grade 5) toxicity occurred in a 32-year-old woman who had received 960 mg/m^2 of BCNU. The BCNU doses had not been properly reduced according to hematologic toxicity guidelines. She developed a life-threatening hematologic toxicity before the onset of pulmonary symptoms. The pulmonary symptoms began 8 months after her surgery. She developed progressive dyspnea, interstitial fibrosis on chest x-ray, and a restrictive pattern on pulmonary function studies. She died of progressive respiratory failure 6 months after BCNU had been discontinued.

The second case of fatal toxicity occurred in a 54-year-old woman who had received 1132 mg/m^2 of BCNU. She was hospitalized with bronchopneumonia 2 weeks prior to her death. Although there were no prior symptoms or signs of BCNU toxicity, pulmonary toxicity was determined to be the cause of death at autopsy. Death occurred 4 months after BCNU had been terminated.

The third case of fatal toxicity occurred in a 41-year-old woman who had received 960 mg/m^2 of BCNU and presented with progressive dyspnea approximately 8 months after surgery. Chest pain, dry hacking cough, clear sputum, and progressive weight loss developed. Pulmonary function tests showed reduced lung volumes and a restrictive pattern. BCNU was discontinued, and within 6 months the toxic effects decreased clinically, only to worsen again at 21 months. Chest x-rays showed increased thickening of the pleura with pleural scarring and basilar fibrosis almost 2½ years later. The patient died of respiratory failure due to pulmonary fibrosis from BCNU, with no evidence of disease.

The patient with grade 3 toxicity developed bilateral spontaneous pneumothoraces requiring chest tubes 11 months after initial brain surgery. Subsequently, bilateral thoracotomies with decortication and a right thoracostomy with abrasion were performed. Biopsies revealed fibrosis compatible with either obstructive pulmonary disease (the patient was a heavy smoker) or BCNU toxicity. Interstitial fibrosis, distended and ruptured alveoli, and bullae were present. At last follow-up, 16 months after initial surgery, the patient was still symptomatic. The occurrence of pneumothoraces had been reported previously in the literature of BCNU lung toxicity. The patient in this series would be at least the sixth reported case (1,3,8).

There were three patients with grade 2 toxicity. One patient developed interstitial pneumonitis confirmed by chest x-ray. The pneumonitis was unresolved at death from glioblastoma 3 months after termination of BCNU. The second patient had interstitial pneumonitis and fibrosis confirmed by pulmonary function tests and gallium scan and died 1 year later from bronchopneumonia and neurologic deterioration. The third patient developed bilateral pulmonary fibrosis but was alive and asymptomatic at last follow-up, 2 years after BCNU termination.

Grade 1 toxicity was established by routine pulmonary function studies in three patients. One patient presented with symptoms of dyspnea and another had mild, easy fatigability. On physical examination, this latter patient had diffuse rhonchi.

A number of factors were analyzed for their potential relationship to BCNU pulmonary toxicity. Characteristics of the patients developing and not developing pulmonary toxicity appear well-balanced with respect to sex, on-study Karnofsky performance score, and prior

TABLE 1.—Patient characteristics and BCNU pulmonary toxicity*

	No pulmonary toxicity established		Pulmonary toxicity established	Total
Total BCNU dose received (mg/m^2)	< 902	≥ 902	≥ 902	
Total No. of evaluable patients	182	97	10	289
Sex				
Male	66	64	60	65
Female	34	36	40	35
Karnofsky performance score				
40–70	41	21	20	34
80–100	59	79	80	66
Age (yrs)				
18–44	16	35	50	24
45–55	29	38	40	32
56–70	55	27	10	44
Associated respiratory disease				
No	97	100	90	97
Yes	3	0	10	3
Associated renal disease				
No	98	99	100	99
Yes	2	1	0	1
Associated liver disease				
No	99	100	100	99
Yes	1	0	0	1
Received misonidazole				
Yes	54	49	30	52
No	46	51	70	48

*Unless otherwise indicated, values = % of patients.

chronic diseases (table 1.) Only one of the ten patients who developed toxicity had documented preexisting lung disease at entry into the study. This patient had tuberculosis and was on treatment with isoniazide. No patients had received chest irradiation.

Receiving misonidazole during the first cycle of chemotherapy did not appear to alter the appearance of pulmonary toxicity. Seven of the ten patients who developed toxicity did not receive misonidazole; the incidence of pulmonary toxicity was 5.0% in those not receiving misonidazole and 2.0% in patients who did receive misonidazole.

Age was also investigated as a potential risk factor for BCNU pulmonary toxicity. The incidence of pulmonary toxicity was 8.7% in patients < 45 years of age, 4.3% in patients between the ages of 45 and 55, and 0.8% in patients ≥ 56 years of age. No patients > 60 years of age developed this toxicity. This observation is directly correlated with survival. In this study (RTOG 79-18), the median survival for patients < 45 years of age was 28 months, compared to 12 months for those age 45–55, and 7 months for those ≥ 56. Table 2 summarizes the incidence of pulmonary toxicity by age for patients receiving ≥ 902-mg/m^2 total BCNU dose.

A proportional hazard regression analysis was performed to investigate the effects of age and treatment with misonidazole (fixed covariates) and BCNU dose (time-dependent covariate) on the development of pulmonary toxicity. Neither age nor treatment with misonidazole was significant. However, increased BCNU dose (after correcting for survival time bias) was significantly associated with increased incidence of pulmonary toxicity ($P < 0.01$). The risk of developing pulmonary toxicity increased from 1.0 at a total BCNU dose of 902 mg/m^2, to 3.30 at 1400 mg/m^2, and to 10.97 at 1900 mg/m^2 (fig 1).

DISCUSSION

A total of 289 patients with malignant glioma who were treated with BCNU as the sole chemotherapeutic agent were evaluated for pulmonary toxicity. No pulmonary toxicity was identified in 182 patients who received < 902-mg/m^2 total BCNU dose. Although fewer patients received larger total doses, the risk of pulmo-

TABLE 2.—BCNU pulmonary toxicity by age in patients receiving ≥ 902-mg/m^2 total BCNU dose*

Age (yrs)	No pulmonary toxicity established	Pulmonary toxicity established	Total
18–44	34 (87.2)	5 (12.8)	39
45–55	37 (90.2)	4 (9.8)	41
56–70	26 (96.3)	1 (3.7)	27
	97 (90.7)	10 (9.3)	107

*Values = No. (%) of patients.

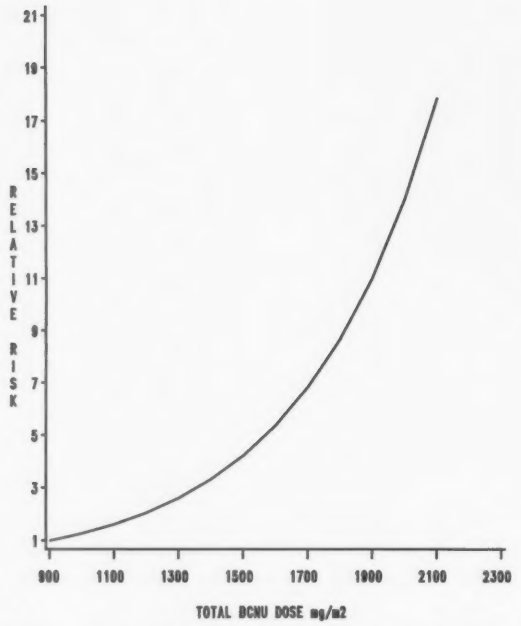

FIGURE 1.—Estimated risk of pulmonary toxicity by total BCNU dose.

nary toxicity in patients who received a total BCNU dose between 1400 and 1900 mg/m^2 was three to 11 times that of patients receiving 902 mg/m^2, respectively. These risks were adjusted for differences in survival time.

The risk of BCNU pulmonary toxicity may be underestimated in this study, as serial diffusion capacity determinations were not routine. Survival in this study of patients with malignant glioma was short (median survival, 12 months) and may have prevented the clinical appearance of latent toxicity. Furthermore, corticosteroids given during radiation therapy and frequently at the time of clinical deterioration may have masked symptoms because of their anti-inflammatory effects.

BCNU pulmonary toxicity was not increased by the concomitant administration of misonidazole, nor were there differences in the risk of BCNU pulmonary toxicity by age.

A recent review of nitrosourea pulmonary toxicity points to the total dose as the single most important determinant of the toxicity (9). Two previous series in the literature, in which BCNU was the only chemotherapeutic agent employed in the treatment of malignant brain tumors, noted an incidence of 20% (19 of 93 patients) and 30% (14 of 47) for pulmonary toxicity (4,6). Each incidence was calculated using a denominator based on total number of treated patients. In the present series, ten of 289 evaluable patients (3.5%) were noted to have developed pulmonary toxicity in a multi-institutional study. In the group restricted to patients receiving at least 902-mg/m^2 total BCNU dose, the unadjusted incidence was ten of 107 (9.3%), with a tendency toward increasing risk with increased total dose in all age groups. This incidence was distinctly lower than that in the two smaller series previously published and may be explained by the absence of routine serial diffusion capacity determinations in the present study and/or the larger drug doses in the smaller series.

Based on the large patient population of the present study and the multivariate regression results, it is recommended that total BCNU dose in any one individual not exceed approximately 1400 mg/m^2. This supports similar recommendations made previously (9). Above this total dose, the incidence of pulmonary toxicity appears to be extremely high. Serial chest x-rays, pulmonary function studies, or diffusion capacities should be done as a baseline, especially in patients who receive $\geq$ 902-mg/m^2 total BCNU dose. Increased vigilance should be maintained in those patients for whom BCNU administration above this level is continued due to clinical necessity.

REFERENCES

1. HOLOYE PY, JENKINS DE, and GREENBERG SD. Pulmonary toxicity in long-term administration of BCNU. Cancer Treat Rep 60: 1691–1694, 1976.
2. CRITTENDEN D, TRANUM BL, and HAUT A. Pulmonary fibrosis after prolonged therapy with 1,3-bis(2-chloroethyl)-1-nitrosourea. Chest 72:372–373, 1977.
3. DURANT JR, NORGARD MJ, MURAD TM, ET AL. Pulmonary toxicity associated with bischloroethylnitrosourea (BCNU). Ann Intern Med 90:191–194, 1979.
4. SELKER RG, JACOBS SA, MOORE PB, ET AL. 1, 3-Bis(2-chloroethyl)-1-nitrosourea (BCNU)-induced pulmonary fibrosis. Neurosurgery 7:560–565, 1980.
5. LIEBERMAN A, RUOFF M, ESTEY E, ET AL. Irreversible pulmonary toxicity after single course of BCNU. Am J Med Sci 279:53–56, 1980.
6. ARONIN PA, MAHALEY MS, JR, RUDNICK SA, ET AL. Prediction of BCNU pulmonary toxicity in patients with malignant gliomas. N Engl J Med 303:183–188, 1980.
7. COX DR. Regression models and lifetables (with discussion). J R Stat Soc Ser B 34:187–220, 1972.
8. WILSON KS, BRIGDEN ML, ALEXANDER S, ET AL. Fatal pneumothorax in "BCNU lung." Med Pediatr Oncol 10:195–199, 1982.
9. WEISS RB, POSTER DS, and PENTA JS. The nitrosoureas and pulmonary toxicity. Cancer Treat Rev 8:111–125, 1981.

Comparative Pharmacokinetics of Doxorubicin and Epirubicin in Patients With Gastrointestinal Cancer[1,2]

Curt Peterson,[3,*] Peter Gunvén,[4] and Nils-Olof Theve[4,5]

The levels of doxorubicin (DOX) and epirubicin (epi-DOX) in serum, tumors, and normal tissues were assayed by high-performance liquid chromatography after peroperative administration of test doses (10 mg) to 14 patients with gastrointestinal cancer. Large interindividual variations were found. The drug levels also varied markedly between tissues from the same patient, with normal liver consistently having the highest drug concentration, followed by tumors and striated muscle, which had similar levels. In serum, a rapid initial distribution phase, with a half-life of about 5 minutes for DOX and 4 minutes for epi-DOX, was followed by a slower decrease in the drug levels. The concentration versus time curves were not influenced by the presence of liver metastases. No metabolites of DOX or epi-DOX were found during the time period studied, in most cases 2 hours after drug administration. Our pharmacokinetic results give no explanation for the claimed superiority of epi-DOX in the treatment of gastrointestinal cancer. [Cancer Treat Rep 70:947-952, 1986]

Epirubicin (epi-DOX) has a wide spectrum of antitumor activity in animal systems. Phase II clinical studies suggest that the drug is possibly less cardiotoxic than its stereoisomer doxorubicin (DOX) (1-3). In addition, there are indications that epi-DOX may be active against cancers in which DOX is ineffective, eg, colorectal carcinoma (2,4,5).

In the present study, we have compared serum and tissue levels of DOX and epi-DOX after iv injections of test doses given peroperatively to 14 patients with gastrointestinal cancer. Tissue samples were taken from tumors and adjacent normal mucosa, from striated muscle (as a substitute for cardiac muscle), and occasionally from other tissues such as liver or metastases, in order to cover the target tissues for the antitumor effect and also some tissues in which important side effects frequently occur.

MATERIALS AND METHODS

Patients

With the approval of the local ethical committee, ten patients who had surgery for gastrointestinal cancer were given a peroperative iv dose of 10 mg of epi-DOX as a rapid injection (< 2 minutes). Four of these patients also received 10 mg of DOX iv immediately before or after epi-DOX, to allow comparisons between the drugs. In addition, four other patients were given only DOX (10 mg). Tissue and serum samples were obtained at various times after injection. For tumors and adjacent normal tissue, the time for interruption of the blood supply to the specimen was considered to be the time of sampling. Nonnecrotic tissue was selected; tissues and sera were stored at −20°C prior to analysis. No patient had renal insufficiency; a few had liver metastases with or without abnormal liver function tests (table 1).

Drug Assay

Serum concentrations of DOX and epi-DOX were determined by high-performance liquid chromatography (6). In brief, a 0.2-ml serum aliquot was added to 0.2 ml of 0.1 M borate buffer (pH 9.8) containing daunorubicin as internal standard. The drugs were extracted with 1.8 ml of chloroform/methanol (4:1 by volume). Separation was performed on a Lichrosorb Si-60 column (Hibar, 25 cm × 4 mm, from E. Merck, Darmstadt, Federal Republic of Germany) eluted with a mixture of chloroform, methanol, glacial acetic acid, and 0.3 mM $MgCl_2$ (720:210:40:30 by volume) at a flow rate of 1.5 ml/minute. The retention time for DOX was about 3.5 minutes and for epi-DOX about 4.5 minutes.

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Sex	Tumor location	Body surface area (m^2)	Karnofsky performance status index	Abnormal liver function tests	Drug given	
							Epi-DOX	DOX
1	63	M	Cecum	2.30	90	+	+	
2	52	F	Rectum	1.78	90	−	+	
3	57	F	Rectum	1.78	90	−	+	
4	69	M	Stomach	1.90	80	−	+	
5	78	F	Rectum	1.45	90	−	+	
6	61	F	Rectum	1.80	90	−	+	+
7	62	M	Rectum	1.80	90	−	+	+
8	66	F	Sigmoid colon	1.62	90	−	+	
9	82	F	Carcinoid	1.70	70	−	+	+
10	81	F	Sigmoid colon	1.62	80	−	+	+
11	66	M	Ascending colon	1.82	70	+		+
12	69	F	Colon (/ovary?)	1.70	70	−		+
13	60	F	Sigmoid colon	1.93	90	−		+
14	55	M	Rectum	2.20	90	+		+

The drugs were quantitated by fluorometry (λ ex 480 nm, λ em 560 nm). The detection limit was about 0.2 pmol, corresponding to a serum concentration of < 0.01 μM. After the tissue specimens were thawed, 2 ml of 0.1 M phosphate buffer, pH 8.1, and 100 μl of 10 μM daunorubicin were added to 0.5–1 g of tissue. The tissue samples were homogenized with two 5-second pulses using a Kinematica polytron at setting 5, followed by sonication for 30 seconds. The drug analysis was then performed as for serum samples.

RESULTS

Serum Concentrations of DOX and Epi-DOX

Very similar biphasic concentration curves were obtained for DOX and epi-DOX (fig 1). The mean initial half-life was about 5 minutes for DOX and 3–4 minutes for epi-DOX, when calculated from curves based on at least two samples within the first 20 minutes after drug administration. After this time, the initial steep decline in the drug concentration seemed to reach an inflexion point. Liver metastases in two DOX-treated cases did not markedly affect the DOX serum concentration curves. One patient had > 50% of the parenchyma involved but only slightly abnormal liver function tests, and the other had less extensive involvement but high levels of alkaline phosphatase (30 μkats/L; normal range, 0.8–4.6). In the case of epi-DOX, one patient with limited liver secondaries and normal liver function tests had an elevated drug serum concentration and retarded elimination. This was, however, also seen in another patient who did not develop liver disease. In four patients given both DOX and epi-DOX, the elimination of the drugs from the blood showed large interindividual variations in the concentration decay curves, but there was little difference between the rate of elimination of the two drugs in each patient.

During the time studied (mostly < 2 hours after the infusion) no reduced drug metabolites could be detected.

Tumor Concentrations of DOX and Epi-DOX

DOX concentrations ranged from 0.13 to 1.5 nmols/g of tissue, and epi-DOX levels ranged from 0.20 to 1.9 nmols/g of tissue (fig 2). Only in one patient, an 82-year-old woman with an unresectable abdominal wall carcinoid, were serial biopsy specimens of the tumor obtained. There was a rapid decline in the tumor concentration of both DOX and epi-DOX in three specimens taken 80, 90, and 100 minutes after drug administration. In all other patients, only one tumor biopsy was done. The time period (40–120 minutes) between drug administration and tumor sampling had no clear effect on the tumor drug concentration. The highest drug levels were seen in the first biopsy specimen of the carcinoid (DOX, 1.5 nmols/g; epi-DOX, 1.9 nmols/g) and in a previously irradiated rectal carcinoma (no DOX given; epi-DOX, 1.7 nmols/g). Two patients with liver metastases had tumor drug levels in the middle range. The ratios between epi-DOX and DOX concentrations in the same biopsy specimen ranged from 0.52 to 1.79 (mean, 1.24).

Liver Concentrations

Normal liver had high drug concentrations. Three patients had DOX levels of 3.7, 3.0, and 2.6 nmols/g of tissue at 30, 80, and 110 minutes after injection, respectively. For epi-DOX, concentrations of 3.7 nmols/g at 50 minutes and 3.1 nmols/g at 55 minutes were obtained. No patient who had had a liver biopsy received both drugs. In neither liver nor any other normal tissue were any reduced metabolites detected during the time studied (occasionally up to > 3 hours).

Muscle Concentrations

DOX levels ranged from 0.04 to 0.40 nmol/g of tissue during the first 2 hours after administration, and the epi-DOX levels were between 0.06 and 0.43 nmol/g (fig

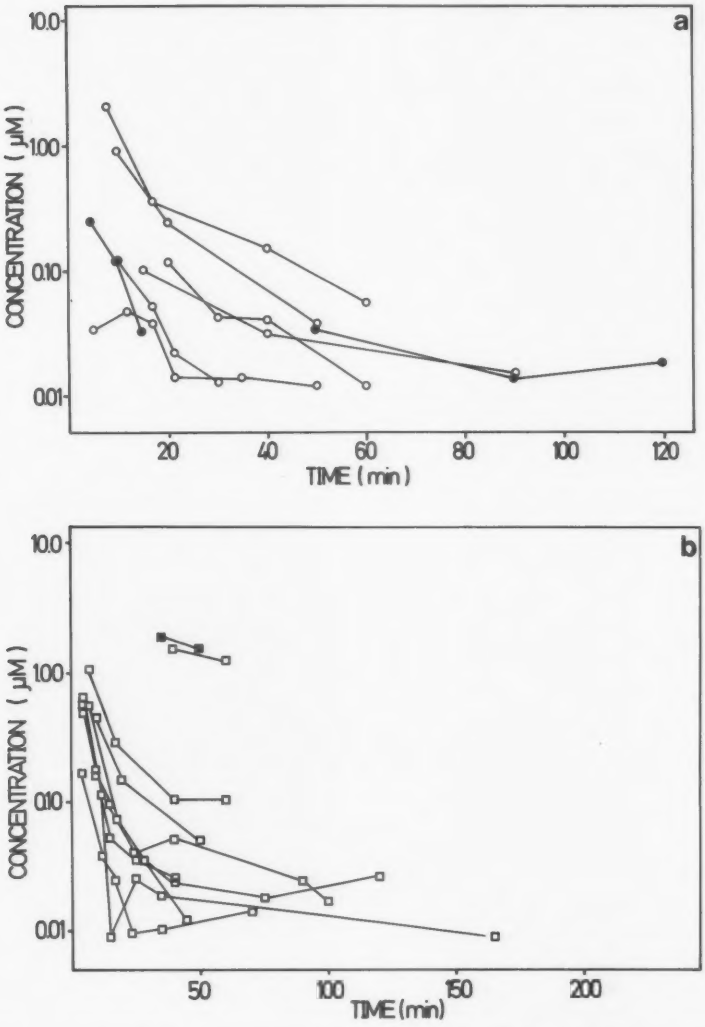

FIGURE 1.—Time courses for the serum concentrations of DOX (a) and epi-DOX (b) after iv injection (< 2 mins) of 10 mg of one or both drugs. Time 0 represents the end of the injection(s). Open symbols show patients with normal liver function tests; solid symbols show those with at least 1 abnormal liver function test result.

3). In contrast to the large interindividual variations, no time trend was evident for either drug. In one case, smooth muscle (myometrium) at 55 minutes had a DOX concentration of 0.07 nmol/g compared to 0.21 nmol/g at the same time in striated muscle. The same biopsy specimens had epi-DOX levels of 0.09 nmol/g (myometrium) and 0.21 nmol/g (striated muscle).

Drug Concentrations in Metastases

In one patient with a cecal cancer, biopsy specimens were obtained from both the primary tumor and a metastasis. The specimen from the primary tumor obtained 120 minutes after the injection of epi-DOX contained 0.58 nmol/g; the liver metastasis sampled 90 minutes later contained 0.68 nmol/g; and a simultaneous muscle specimen contained 0.49 nmol/g. The epi-DOX level in a lymph node metastasis from a rectal cancer was 0.51 (muscle, 0.20) nmol/g at 40 minutes, but there was no sample from the primary tumor. Serial biopsy specimens of omental deposits from a tumor later classified as an ovarian carcinoma had DOX levels from 0.46 to 0.55 nmol/g between 15 and 60 minutes after the injection of DOX. These values were

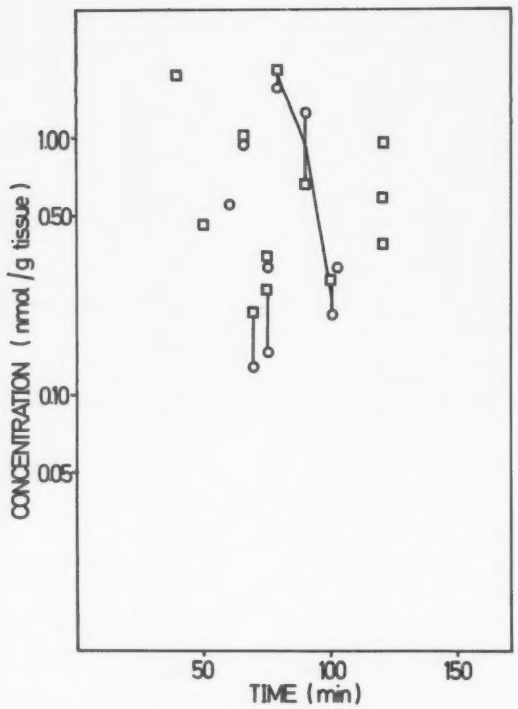

FIGURE 2.—Concentrations of DOX (circles) and epi-DOX (squares) in primary tumor biopsies obtained at various times after iv injection of 10 mg of the drugs. Connected squares and circles indicate samples from patients given a mixture of DOX and epi-DOX. In 1 patient, 3 serial biopsy specimens were obtained. In all others, a single biopsy specimen was obtained.

slightly higher than those obtained in simultaneous muscle biopsies, but no primary tumor was available for comparison. A liver tumor secondary from a rectal cancer had a DOX level of 0.39 nmol/g at 25 minutes, as compared to 0.08 nmol/g in skeletal muscle at the same time, but again no biopsy specimen could be obtained from the primary tumor.

Drug Levels in Other Normal Tissues

Adjacent normal mucosa was usually sampled from resected stomachs or intestines. The tumor/mucosa drug concentration ratio ranged from 0.5 to 2.0 (mean, 1.1) for DOX; for epi-DOX the range was from 0.53 to 2.6 (mean, 1.3).

Tumor/Muscle Ratios of the Drugs

These ratios were calculated by comparing the tumor drug levels with those in simultaneously biopsied muscle or with drug levels interpolated from the concentration curve for serial muscle biopsies. The ratios did not differ with the time after drug administration (correlation coefficients for ratio versus time were 0.17 for DOX and 0.20 for epi-DOX, both not significantly different from 0 so that group mean ratios were calculated and compared by Student's t-test). The group mean ratio for DOX was 3.5 ± 1.8, and for epi-DOX it was 3.1 ± 0.5 ($P > 0.05$).

DISCUSSION

Preliminary studies indicate that, in contrast to DOX, epi-DOX may have some activity against colorectal cancer (2,4,5). One possible explanation for this difference could be a higher uptake and retention of epi-DOX in the tumor tissue. We have therefore compared serum and tissue levels of DOX and epi-DOX in patients with gastrointestinal tumors. In addition to tumor tissue, we also sampled normal adjacent mucosa, striated abdominal muscle (as a possible substitute for myocardium), and other appropriate tissues, such as metastases and normal liver.

There were pronounced interindividual differences in the drug concentrations not only in tumors and metastases but also in serum and normal tissues. Ten mg of DOX and/or epi-DOX was given to all patients regardless of body weight or surface area. However, there was no apparent relationship between the drug concentrations in serum and tissues and the body surface area of the individual patient.

Nonnecrotic and homogeneous tissues without contaminating fat or connective tissue were selected for sampling. Nevertheless, some anomalies were observed, eg, in the curves for drug concentrations in muscle. These showed increases, decreases, or unchanged levels during the same time interval in different patients. It is possible that this can be explained by differences in the blood content of the biopsy specimens, or by changes in the extracellular pH during inhalation anesthesia, with subsequent changes in the ionization of the drugs.

There was no obvious variation in the drug levels in the tumors with time after administration during the relatively short time interval studied (35–120 minutes). Likewise, the muscle concentrations during the same time also did not show any clear time trends. Therefore, we calculated tumor/muscle ratios regardless of time after drug administration, in an attempt to describe the conditions in the target tissues for the antitumor activity and for the dose-limiting cardiomyopathy, with necessary reservations for the use of a substitute for myocardium. The tumor concentration of epi-DOX was slightly higher than that of DOX in five of six biopsies in which both drugs were assayed. In the muscle biopsies in which both drugs were quantitated, epi-DOX levels were an average of 1.6 times higher than those of DOX. On an individual basis, the tumor/muscle drug level ratios were slightly lower for epi-DOX than for DOX, although the difference was not statistically significant.

Serum DOX concentration curves followed previously described distribution and elimination phases (7), although a third phase could not be detected due to our

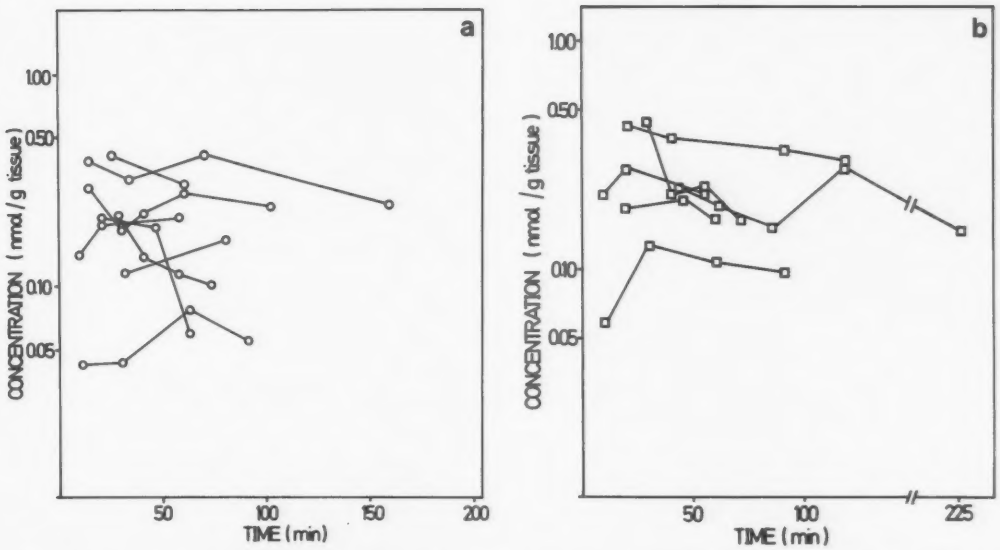

FIGURE 3.—Concentrations of DOX (a) and epi-DOX (b) in biopsies of skeletal muscle obtained at various times after iv injection of 10 mg of the drugs. Connected symbols indicate serial biopsies obtained from the same patient.

relatively short observation period. In our study, the few patients with liver metastases did not differ from the others. The small number of such patients makes it difficult to relate this finding to reports of delayed plasma clearance of DOX with elevated plasma levels both initially and 2.5–5 hours after infusion (8), or to the results of another study in which a patient with liver tumors but normal bilirubin levels had normal DOX clearance (7).

Patients with a slow decline of the serum DOX concentrations tended to have relatively low tumor concentrations. This is compatible with a distribution phase where tumor uptake may be an indicator of the uptake in other tissue compartments as well, possibly being influenced by a common factor eg, pH. In our study, the half-life for DOX in the initial phase (about 5 minutes) was approximately the same as that reported by Gil et al (9).

The estimated plasma half-life for epi-DOX in the present study (3–4 minutes) can be compared to the results of another study giving values of 4.8 minutes for epi-DOX and 4.6 minutes for DOX (10). The small number of patients in our study and the considerably higher dose of 75–90 mg/m^2 in the quoted report seem sufficient to explain the small difference in half-life.

Our failure to detect reduced metabolites of DOX or epi-DOX can also be explained by the low dose which allows drug determination only during a short time period.

Concentrations of DOX and epi-DOX varied considerably between different tissues in both our study and studies by other investigators (3,7,8,11). The liver consistently had high levels of both drugs and the levels in tumors were markedly lower. A report on a single case of colonic carcinoma with very high tissue levels of DOX (7) contrasts to our findings. The discrepancy may be related to our selection of nonnecrotic parts of the tumors, since these have lower DOX levels than necrotic areas (12). Similarly, radiation-induced hyperemia or tissue damage may explain why the highest concentration found in a colorectal carcinoma biopsy was obtained in a preoperatively irradiated rectal tumor, while other colonic and rectal tumors had completely overlapping DOX and epi-DOX levels.

It is not clear to what extent skeletal muscle can be used as a model for cardiac muscle with regard to anthracycline uptake, retention, and effects. The drugs have a high affinity to DNA, and the molar concentration of DNA in myocardium, at least in rats and rabbits, is about ten times that in skeletal muscle (13). Similarities between DOX-induced morphological changes in myocardium and skeletal muscle have, in fact, suggested that skeletal muscle may be used as a model for myocardium in this context (14). In the absence of possibilities to assay both drug levels and damage in serial myocardial biopsies in patients, this will remain a matter of uncertainty.

A further source of uncertainty when attempting to relate myocardial to skeletal muscle uptake of anthracyclines is the time after drug administration. In one study (15), heart DOX levels in rats were higher than skeletal muscle concentrations at 3 hours, but approached the skeletal muscle concentrations at 12–48 hours.

In our study the tumor/muscle concentration ratio was slightly lower for epi-DOX than for DOX. This makes it unlikely that the observed decreased cardiotoxicity of epi-DOX is simply related to its muscle concentration. Intracellular retention of active drug and qualitative difference between myocardium and skeletal muscle must obviously be considered. Also, the claimed superiority of epi-DOX to DOX in the treatment of colorectal cancer cannot be explained by strikingly different tumor uptakes of the two drugs.

REFERENCES

1. KOLARIC K, POTREBICA V, and CERVEK J. Phase II clinical trial of 4′-epi-doxorubicin in metastatic solid tumors. J Cancer Res Clin Oncol 106:148–152, 1983.
2. FERRAZZI E, NICOLETTO O, VINANTE O, ET AL. Phase II study of 4′-epi-doxorubicin. Tumori 68:431–435, 1982.
3. BONADONNA G, and BONFANTE V. Clinical evaluation of 4′-epi-doxorubicin and 4-demethoxy daunorubicin. *In* Anthracycline Antibiotics in Cancer Therapy (Muggia FM, Young CW, and Carter SK, eds). Developments in Oncology 10, Dordrecht, The Netherlands, Martinus Nijhoff, 1982, pp 455–465.
4. FALKSON G, FALKSON HC, VOROBIOF DA, ET AL. A phase II study of 4′-epi-doxorubicin (IMI 28) in colorectal cancer. *In* Proceedings of the 13th International Congress of Chemotherapy (Spitzy KH, and Karrer K, eds). Vienna, 1983, Part 215, pp 31–35.
5. FOSSÅ SD. 4′-Epi-doxorubicin in advanced renal cell carcinoma and colorectal cancer. *In* Proceedings of the Symposium Anthracyclines and Cancer Therapy (Hansen HH, ed). Ronneby Brunn, Sweden, Oct 6–7, 1982. Excerpta Medica, 1983, pp 231–236.
6. PETERSON C, PAUL C, and GAHRTON G. Anthracycline DNA-complexes as slow-release preparations in the treatment of acute leukemia. *In* Controlled Release of Pesticides and Pharmaceuticals (Lewis DH, ed). New York, Plenum Press, 1981, pp 49–65.
7. CHAN KK, COHEN JL, GROSS JF, ET AL. Prediction of adriamycin disposition in cancer patients using a physiologic, pharmacokinetic model. Cancer Treat Rep 62:1161–1171, 1978.
8. LEE Y-TN, CHAN KK, HARRIS PA, ET AL. Distribution of adriamycin in cancer patients. Tissue uptakes, plasma concentration after iv and hepatic ia administration. Cancer 45:2231–2239, 1980.
9. GIL P, FAVRE R, DURAND A, ET AL. Time dependency of adriamycin and adriamycinol kinetics. Cancer Chemother Pharmacol 10:120–124, 1983.
10. WEENEN H, LANKELMA J, PENDERS PGM, ET AL. Pharmacokinetics of 4′-epi-doxorubicin in man. Invest New Drugs 1:59–64, 1983.
11. BROGGINI M, COLOMBO T, MARTINI A, ET AL. Studies on the comparative distribution and biliary excretion of doxorubicin and 4′-epi-doxorubicin in mice and rats. Cancer Treat Rep 64:897–904, 1980.
12. PIAZZA E, NATALE N, TRABATTONI A, ET AL. Plasma and tissue distribution of adriamycin in patients with pelvic cancer. Tumori 67:533–537, 1981.
13. TERASAKI T, IGA T, SUGIYAMA Y, ET AL. Experimental evidence of characteristic tissue distribution of adriamycin. Tissue DNA concentration as a determinant. J Pharm Pharmacol 34:597–600, 1982.
14. MERSKI J, DASKAL Y, and BUSCH H. Comparison of adriamycin-induced nucleolar segregation in skeletal muscle, cardiac muscle, and liver cells. Cancer Treat Rep 62:771–778, 1978.
15. WILKINSON PM, and MAWER GE. The persistence of adriamycin in man and rat. Br J Clin Pharmacol 1:241–247, 1974.

Effects of Mega-COMLA (Cyclophosphamide, Cytarabine, Vincristine, and Methotrexate Followed by Leucovorin and Prednisone) Plus CHOP (Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone) in the Treatment of Lymphoid Neoplasms With Very Poor Prognosis[1,2]

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Treatment results remain very poor for some clinical and histopathologic subsets of patients with aggressive non-Hodgkin's lymphoma. We treated 21 such patients with a high-dose combination chemotherapy regimen [Mega-COMLA (cyclophosphamide, cytarabine, vincristine, and methotrexate followed by leucovorin and prednisone) + CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)] in an attempt to improve disease-free survival. Neoplasms were classified using the Lukes-Collins system. Eight patients had T-cell lymphomas (convoluted lymphocytic lymphoma, four patients; T-cell lymphoma/leukemia, one; and peripheral T-cell lymphoma, three), eight had B-cell lymphomas (immunoblastic sarcoma, five patients; small noncleaved follicular center cell, one; and large noncleaved follicular center cell, two), and five had nontypable large noncleaved cell lymphomas. All patients were previously untreated; 18 of 21 patients had clinical stage III or IV disease. Following induction therapy (4–8 weeks' duration), 16 patients (76%) achieved complete remission, while three had partial remission. Two patients died of sepsis during induction therapy. Eleven of 16 complete responders (69%) remain in complete remission after a median follow-up of 35 months. The actuarial 3-year survival rate is 51% for the entire group. Myelosuppression with this regimen was severe and prolonged, with a median duration of neutropenia (< 500 cells/μl) of 14 days. Seven patients (33%) developed severe neuropathy following induction treatment. High-dose induction therapy with this regimen resulted in a high complete remission rate with manageable toxicity. Survival results are encouraging when compared retrospectively to our patients with similar poor-prognosis histologies treated with standard combination chemotherapy. However, the value of this intensive therapy, relative to newer ("third-generation") regimens, can only be established by prospective randomized studies. [Cancer Treat Rep 70:953–958, 1986]

During the last 10 years, treatment of high-grade non-Hodgkin's lymphoma has greatly improved. Presently, between 50% and 70% of patients with advanced diffuse histiocytic lymphomas (stage III or IV) can be cured of their malignancy with appropriate combination chemotherapy (1–6).

As effective chemotherapy regimens have developed, various clinical and histopathologic features have been identified which predict poor long-term survival following treatment. Clinical characteristics associated with poor prognosis include involvement of certain organs with lymphoma (bone marrow, central nervous system, and liver) or the presence of very large intra-abdominal masses (>10 cm) with gastrointestinal involvement (1,7,8).

Burkitt's lymphoma and lymphoblastic (convoluted lymphocytic) lymphoma are well-recognized, distinct clinicopathologic entities with relatively poor prognosis when treated with standard approaches (9–11). Immunologic subtyping of high-grade lymphomas, which is integral to the Lukes-Collins classification system (12), has resulted in the identification of several other clini-

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copathologically distinct neoplasms. Routine use of the Lukes-Collins classification system has enabled us to identify poor-prognosis subsets of high-grade lymphomas which are traditionally included in the heterogeneous "diffuse histiocytic lymphoma" group of Rappaport. These groups include peripheral T-cell lymphomas (immunoblastic sarcomas of T cells), immunoblastic sarcomas of B cells, and small noncleaved follicular center cell (FCC) lymphomas of B cells (13–15).

Limited evidence already exists that treatment results can be improved in certain poor-prognosis lymphomas by using chemotherapeutic regimens of increased intensity. Cure rates of Burkitt's lymphoma and lymphoblastic lymphoma have been improved using this approach (11,16–21). The use of outpatient regimens of increased intensity has improved the outlook of patients with poor prognostic clinical features (eg, tumor involvement of bone marrow and liver) (5,6). In addition, some patients with lymphomas resistant to standard therapy have been cured with high-dose cyclophosphamide and total-body irradiation followed by autologous bone marrow transplantation (22). However, these reports concern limited numbers of patients, and results of intensive therapy have not been reported in other poor-prognosis subgroups recognized by the Lukes-Collins classification system.

In an attempt to improve treatment results in the subsets of high-grade lymphoma with very poor prognosis, we designed an intensive, inpatient combination chemotherapy regimen (Mega-COMLA; cyclophosphamide, cytarabine, vincristine, and methotrexate followed by leucovorin and prednisone) based on the previously reported COMLA regimen (2). Patients were eligible for treatment with this regimen if they had clinical and/or pathologic features which predicted a poor outcome (< 30% long-term survival) with standard outpatient combination chemotherapy regimens.

MATERIALS AND METHODS

Between June 1982 and August 1984, 21 previously untreated patients with biopsy-proven malignant lymphoma were treated with the Mega-COMLA regimen. Histologic sections from all patients were reviewed at Vanderbilt, and immunologic studies (cell suspension immunotyping and/or tissue immunoperoxidase studies) to define the cell of origin were performed in all patients (cell suspension immunotyping, 15 patients; immunoperoxidase studies, 17 patients). Patients with the following diagnoses (Lukes-Collins classification system) were eligible: convoluted lymphocytic (thymic) lymphoma; peripheral T-cell lymphoma; immunoblastic sarcoma of B cells; and small noncleaved FCC lymphoma (Burkitt's or non-Burkitt's type). In addition, patients with large noncleaved FCC lymphoma were eligible if they had one or more of the following poor prognostic clinical features: bone marrow involvement; CNS involvement; hepatic involvement; or abdominal masses > 10 cm in diameter involving the gastrointestinal tract. Patients with large noncleaved cell lymphomas who did not have any of the above clinical features were excluded, as were all patients with large cleaved cell lymphomas.

Routine staging procedures included physical examination, chest roentgenogram, computerized tomographic scans of the chest and abdomen, bone marrow aspiration and biopsy, and lumbar puncture.

Table 1 shows the clinical and pathologic features of our 21 patients. The median age was 43 years (range, 18–68); 14 patients were male and seven were female. The histologic diagnoses were as follows: T-cell lymphoma, eight patients (convoluted lymphocytic, four; peripheral T-cell lymphoma, three; and T-cell lymphoma/leukemia, one); immunoblastic sarcoma of B cells, five; small noncleaved FCC lymphoma, one; large noncleaved FCC lymphoma, two; and large noncleaved lymphoma untypable for B or T cells, five. All patients with large transformed cell lymphomas (either of B-cell origin or untypable) had poor prognostic clinical features. Fourteen patients had stage IV disease, while five had stage III and two had stage II lymphomas.

Mega-COMLA Chemotherapy Regimen

The Mega-COMLA regimen consisted of two intensive inpatient cycles of therapy given at 28-day intervals, as shown in figure 1: cyclophosphamide (1500 mg/m^2 by iv bolus over 30–60 minutes on Days 1 and 2); cytarabine (100 mg/m^2/24 hours by continuous iv infusion for 5 days); vincristine (1.4 mg/m^2 by rapid iv bolus on Days 1, 8, 15, and 22); and methotrexate (200 mg/m^2 by iv bolus on Days 8 and 22) followed by leucovorin (15 mg/m^2 iv or orally every 6 hours × eight doses beginning 24 hours after methotrexate) and prednisone (60 mg/m^2 orally on Days 1–14). The vincristine dose was modified to 1.4 mg/m^2 by iv push on Days 1 and 15 and the maximum dose was limited to 2.0 mg after the first six patients were treated, due to the development of unexpectedly severe peripheral neuropathy in three patients.

Following induction therapy, patients had complete restaging including repetition of all initial staging procedures. Three patients had rebiopsy of residual abnormalities of questionable significance (lymph nodes, two patients, and breast mass, one). Patients with normalization of all studies were classified as complete responders, while patients with decrease in tumor size > 50% but who still had residual lymphoma documented at restaging were classified as partial responders. Patients with residual tumor were considered treatment failures and removed from the study.

Patients in complete remission after induction therapy were scheduled to receive six cycles of outpatient chemotherapy with CHOP [cyclophosphamide (750 mg/m^2 by iv bolus), doxorubicin (50 mg/m^2 by iv bolus), vincristine (1.0 mg/m^2 by iv bolus), and prednisone (100 mg orally every day × 5)]. Cycles were repeated every 28 days.

Ten patients considered to be at high risk for CNS relapse received CNS prophylaxis. High-risk groups included the following: (*a*) patients with T-cell lympho-

TABLE 1.—Clinical features and response to therapy of 21 patients treated with Mega-COMLA

Patient No.	Age (yrs)	Sex	Histology	Clinical stage	Extranodal sites of involvement	Response*	Duration (mos)	Survival (mos)
1	25	M	Large noncleaved, untypable lymphoma	II B		CR	33+	33+
2	54	M	Peripheral T-cell lymphoma (HTLV+)	II A_E	25 × 25-cm chest wall mass	CR	35+	35+
3	60	M	Peripheral T-cell lymphoma	III A	Spleen	CR	22+	22+
4	31	M	Convoluted lymphocytic lymphoma	III B	Spleen	CR	43+	43+
5	18	M	Convoluted lymphocytic lymphoma	III A	18 × 20-cm mediastinal mass	CR	36+	36+
6	68	M	Immunoblastic sarcoma, B-cell	IV A	Bone marrow	CR	31+	31+
7	26	F	Acute lymphoblastic leukemia/lymphoma, T-cell		Bone marrow	CR	35+	35+
8	53	M	Immunoblastic sarcoma, B-cell	IV A	Salivary gland, soft tissue/ muscle (neck), lung	CR	35+	35+
9	63	M	Large noncleaved, untypable lymphoma	IV B	Liver, bone marrow	CR	32+	32+
10	40	M	Small noncleaved, B-cell lymphoma	IV A	Bone	CR	43+	43+
11	49	F	Immunoblastic sarcoma, B-cell	IV B	Bone marrow	CR	42+	42+
12	43	M	Large noncleaved, untypable lymphoma	III A	Spleen	CR	10	26
13	26	F	Convoluted lymphocytic lymphoma	IV B	Pleura, pericardium, ? lung	CR	7	10
14	57	M	Convoluted lymphocytic lymphoma	IV A	Bone marrow	CR	6	10
15	26	F	Large noncleaved, untypable lymphoma	IV B	Lungs, kidneys, ovaries	CR	10	23
16	40	M	Large noncleaved, B-cell lymphoma	IV A	Bone marrow	CR	22	25
17	60	M	Immunoblastic sarcoma, B-cell	IV B	Bone, chest wall	PR		9
18	20	M	Peripheral T-cell lymphoma	IV B	Pleura, ascites, bone marrow	PR		14
19	18	F	Large noncleaved, B-cell lymphoma	II A	12 × 12-cm mediastinal mass	PR		19
20	63	F	Immunoblastic sarcoma, B-cell	IV B	Kidneys, gastrointestinal tract, pleura	Treatment-related death		1
21	50	F	Large noncleaved, untypable lymphoma	IV A	Ovaries, omentum, pleura	Treatment-related death		2

*CR = complete response; PR = partial response.

mas; (*b*) patients with small noncleaved FCC lymphomas; and (*c*) patients with bone marrow involvement. CNS prophylaxis began at Week 7 of induction therapy and consisted of intrathecal methotrexate (12 mg of methotrexate administered twice weekly for five doses) and whole-brain irradiation (2000 rads in ten fractions).

Disease-free survival was calculated from the day complete remission was documented using the actuarial method of Kaplan and Meier (23). Patients who died as a result of toxicity are included as treatment failures.

RESULTS

Fifteen patients received two cycles of high-dose induction therapy, while six received only one cycle. One patient died of probable sepsis during the first induction cycle, while five did not receive the second cycle due to complications (severe infection during first cycle, three patients; prolonged thrombocytopenia following first cycle, one; and acute myocardial infarction between Cycles 1 and 2, one).

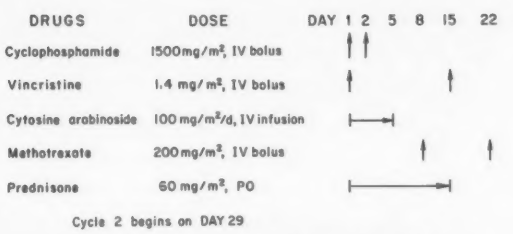

FIGURE 1.—Mega-COMLA induction regimen.

Sixteen of 21 patients (76%) achieved complete remission following induction therapy (table 1). Fifteen of these 16 patients subsequently received CHOP therapy; one patient received no further therapy due to disseminated cytomegalovirus infection. Following the intensive induction therapy, full doses of CHOP were difficult to administer, due to myelosuppression. Because of this problem, only four patients received the planned six cycles, while 12 received fewer doses (four cycles, ten patients; two cycles, one; and one cycle, one). Five of 16 patients (31%) have relapsed from complete remission; four relapsed at multiple sites, while one had an isolated CNS recurrence. This patient (who had not received CNS prophylaxis) was treated with intrathecal methotrexate and cranial irradiation with good response, but subsequently had a systemic recurrence 9 months after CNS recurrence. Relapses occurred during CHOP therapy or within 3 months of its completion in three of five patients. The number of cycles of CHOP therapy received was unrelated to relapse rate or survival.

Three patients (14%) had partial response to Mega-COMLA therapy. These three patients died of progressive lymphoma 9, 14, and 19 months after diagnosis.

The overall 3-year actuarial survival from the time of completion of induction therapy was 52% (fig 2). Eleven patients are relapse-free at a median of 35 months after diagnosis (range, 22–43 months); ten of these 11 patients have been followed for > 24 months following diagnosis and are considered unlikely to relapse.

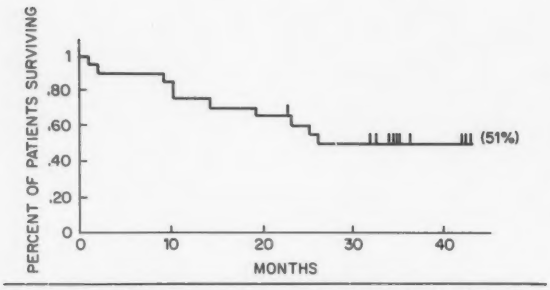

FIGURE 2.—Actuarial relapse-free survival curve for 21 patients treated with Mega-COMLA. 3-yr actuarial survival rate is 50%. (Disease-free survivors are represented by tick marks.)

Disease stage at diagnosis was not a useful predictor of treatment outcome. Both complete response rate and relapse-free survival were comparable in stage II, III, and IV patients (table 2). Likewise, complete response rates and long-term survival rates were comparable among the various histologic subgroups (table 2).

As expected, myelosuppression during the induction phase of treatment was the major toxic effect and was severe in all patients. All patients were hospitalized during high-dose induction therapy; the median hospital stay was 21 days/cycle. The median duration of severe neutropenia (wbc count $< 500/\mu l$) was 14 days (range, 8–32), while the median duration of severe thrombocytopenia (platelet count $< 20{,}000/\mu l$) was 6 days (range, 4–24). Infections or fever occurred during each of the 36 induction cycles: fever with negative cultures, 23 cycles; bacteremia/sepsis, 11 (*Staphylococcus epidermidis*, five; *Pseudomonas aeruginosa*, two; *Escherichia coli*, two; and multibacterial, two); fungemia, one (*Candida albicans*, one); *Staphylococcus aureus* cellulitis, one; disseminated cytomegalovirus, one; and hepatitis B, one. There were two treatment-related fatalities (9%), both due to bacterial sepsis during severe neutropenia.

Seven patients (33%) developed severe peripheral neuropathy during the first 3 months of therapy. These patients each had peroneal nerve palsies with foot drop and were unable to ambulate for several months following induction therapy. Peripheral neuropathy has slowly and completely resolved in all of the long-term survivors who experienced this complication. Three patients (14%) developed hemorrhagic cystitis, which was transient and not a severe clinical problem. Alopecia occurred in all patients; mild to moderate nausea and vomiting occurred during high-dose cyclophosphamide therapy.

DISCUSSION

Although treatment of high-grade non-Hodgkin's lymphomas has improved markedly, certain subgroups of these lymphomas continue to do poorly when treated with "standard" outpatient chemotherapy combinations. In an attempt to improve survival in these subgroups with very poor prognosis, we developed an intensive inpatient combination regimen. Our induction regimen is an intensification of the COMLA regimen, which has been effective in the outpatient treatment of high-grade lymphomas (2). Our modifications of the COMLA regimen are as follows: (*a*) the doses of cyclophosphamide and methotrexate in the induction regimen have been doubled; (*b*) the interval between cyclophosphamide doses has been reduced from 85 to 28 days; (*c*) cytarabine is given by continuous infusion, a pharmacologically more rational way of administering this drug than the sc bolus injections at 1-week intervals used in the COMLA regimen; (*d*) prednisone has been added to the induction regimen; and (*e*) patients achieving complete remission with induction therapy received additional outpatient therapy with a doxorubicin-containing combination (CHOP).

As expected, myelosuppression with this induction regimen was severe and prolonged, but was manageable in most patients in a closely monitored inpatient setting. The 9% overall mortality rate we experienced as a result of sepsis is similar to the mortality rate reported with the more intensive outpatient combination regimens (4,5). The development of severe peripheral neuropathy

TABLE 2.—Response to Mega-COMLA therapy according to disease stage and histology

	No. of patients	Response		Remains in complete response	Early death
		Partial	Complete		
Stage					
II	3	1	2 (70%)	2 (67%)	0
III	4	0	4 (100%)	3 (75%)	0
IV	14	2	10 (71%)	6 (43%)	2 (14%)
Histology					
T-cell lymphoma	8	1	7 (88%)	5 (63%)	0
Immunoblastic sarcoma, B-cell	5	1	3 (60%)	3 (60%)	1 (20%)
Small noncleaved, B-cell lymphoma	1	0	1 (100%)	1 (100%)	0
Large noncleaved, untypable lymphoma	5	0	4 (80%)	2 (40%)	1 (20%)
Large noncleaved, B-cell lymphoma	2	1	1 (50%)	0 (0%)	0

in seven patients was unexpected and seemed out of proportion to the amount of vincristine administered. Four patients developed severe neuropathy following induction therapy even after the frequency and maximum dose of vincristine during the first 8 weeks of treatment were reduced. The combination of drugs administered simultaneously may have acted synergistically to produce this toxicity. Overall, we feel the administration of this induction regimen is feasible from a toxicity standpoint; further reduction in the frequency of vincristine administration would probably reduce neurotoxicity.

Recent progress in the ability to characterize lymphomas pathologically has led to controversy regarding the classification, with resulting confusion regarding the prognosis of different subgroups. Burkitt's lymphoma and lymphoblastic (convoluted lymphocytic) lymphoma are now well-recognized clinical entities, for which intensification of chemotherapeutic treatments has resulted in improved therapeutic results (10,15). Table 3 shows the ability of increasingly intensive combination chemotherapy regimens to produce increased numbers of long-term survivors in these patient groups.

Increasing evidence documents the ability of the Lukes-Collins classification system to define patient subgroups with very poor prognosis when treated with standard ("second-generation") combination chemotherapy regimens. We recently reported retrospectively our experience with peripheral T-cell lymphomas and small noncleaved cell lymphomas treated with such regimens (13,14). In both of these groups, complete response rates were low (24% and 29%, respectively), and long-term survival was achieved by $< 20\%$ of the patients in each group. Similar results were reported by Levine et al (15) in the treatment of immunoblastic sarcomas of B cells and peripheral T-cell lymphomas. In addition, reclassification of the patients in the original COMLA series (2) according to the Lukes-Collins system has revealed a marked difference in therapeutic results between B-cell and non-B-cell lymphomas (24). Patients with B-cell lymphomas (primarily FCC lymphomas) had a higher complete response rate (64% vs 38%) and more relapse-free survivors (49% vs 13%) than did patients with non-B-cell lymphomas (ie, T-cell or unclassifiable lymphomas). Newcomer et al (25) also reported inferior results in patients with immunoblastic sarcoma versus those with other large cell lymphomas (42% vs 82% actuarial 3-year disease-free survival, respectively) when treated with either the ACOMLA (COMLA + doxorubicin) or CHOP-B (CHOP + bleomycin) regimen. Although all of these studies are retrospective and include relatively small numbers of patients, the poor treatment results in these patient groups are consistent.

Recently, several new ("third-generation") combination chemotherapy regimens (eg, PROMACE-MOPP, MACOP-B, and M-BACOD) have been reported to improve results in the treatment of patients with high-grade non-Hodgkin's lymphomas (4–6). In addition to improving overall complete response and long-term survival rates, patients with clinical features previously associated with poor therapeutic outcome, including bone marrow involvement, liver involvement, and bulky intra-abdominal disease, no longer had long-term survival rates which were significantly inferior (4,5). The value of cell-surface phenotyping as a prognostic indicator is also currently unclear, since therapeutic outcome with third-generation regimens has been similar in the B- and T-cell "large cell" lymphomas (5,6). In addition, Klimo and Connors (6) reported complete remissions in 15 of 17 patients with immunoblastic sarcoma (cell phenotype unspecified) treated with MACOP-B and noted no increased rate of relapse in this group.

The efficacy of the Mega-COMLA regimen in compar-

TABLE 3.—Improved efficacy of intensive chemotherapy in the treatment of Burkitt's lymphoma and lymphoblastic lymphoma

Investigator(s), ref No., yr	No. of patients	Regimen*	No. of complete responders (%)	No. of long-term survivors (%)
		Burkitt's lymphoma		
Ziegler, 16, 1978	27	COM	27 (100%)	14 (52%)
Magrath et al, 17, 1984	37	CHOMP	32 (86%)	24 (65%)
Sullivan and Ramirez, 18, 1985	24	HDCTX/HDMTX, Ara-C/VCN/ Prednisone	22 (92%)	18 (75%)
		Convoluted lymphocytic (lymphoblastic) lymphoma		
Rosen et al, 9, 1978	12	CVP, CHOP	NA	2 (17%)
Coleman et al, 19, 1981	13	CHOP, HDMTX, L-asp	13 (100%)	8 (62%)
Levine et al, 20, 1983	15	Modified LSA-L2	11 (73%)	7 (47%)
Weinstein et al, 21, 1983	21	APO	20 (95%)	14 (66%)
Magrath et al, 17, 1984	14	CHOMP	14 (100%)	10 (69%)

*COM = cyclophosphamide, vincristine, and methotrexate; CHOMP = COM + doxorubicin and prednisone; HDCTX = high-dose cyclophosphamide; HDMTX = high-dose methotrexate; Ara-C = cytarabine; VCN = vincristine; CVP = cyclophosphamide, vincristine, and prednisone; L-asp = L-asparaginase; LSA-L2 = multidrug regimen containing cyclophosphamide, vincristine, doxorubicin, prednisone, cytarabine, 6-thioguanine, L-asparaginase, and lomustine; and APO = doxorubicin, prednisone, and vincristine (also includes L-asparaginase, 6-mercaptopurine, and methotrexate in consolidation phase).

ison to other recently reported intensive outpatient chemotherapy regimens is unclear, since a direct comparison has not been performed. Indeed, very few randomized trials comparing treatments for high-grade non-Hodgkin's lymphoma have been conducted, and the relative efficacy of many published regimens is unknown. Certainly, patient selection can influence therapeutic results (26). In designing this study, we were careful to select patients from the group of high-grade non-Hodgkin's lymphomas who had either clinical or histologic features which had previously been recognized to be associated with poor prognosis. Most of our patients not only had high-stage tumors, but also had histologic subtypes previously associated with poor prognosis. Treatment results using third-generation combination regimens in a group of patients with these poor prognostic features have not been reported; we suspect that long-term survival rates in this group would be lower than those previously reported. We therefore feel our achievement of a 51% long-term survival rate with Mega-COMLA is encouraging; however, resolution of these questions awaits a randomized comparison of these regimens.

Our initial experience with the Mega-COMLA regimen has suggested several modifications which may reduce toxicity and increase effectiveness. Modification of the vincristine dosing schedule may minimize peripheral neuropathy. The occurrence of tumor relapse during or immediately following CHOP maintenance therapy suggests that this type of maintenance may be ineffective following initial intensive therapy. Maintenance therapy of any type may be unnecessary in high-grade lymphomas when initial therapy is very intensive. The possible exception is thymic lymphoma in that we observed two relapses among four patients who achieved complete remission; other studies have suggested a role for maintenance therapy in this subset of high-grade lymphoma which is closely related to T-cell acute lymphocytic leukemia (20). The incorporation of other agents with known activity against lymphoma (anthracyclines and etoposide) into a very intensive induction regimen may further improve the cure rate while allowing a reduced duration of therapy. Final proof of the efficacy of this approach will await comparison with standard combination chemotherapy in randomized trials.

REFERENCES

1. SCHEIN PS, DEVITA VT, JR, HUBBARD S, ET AL. Bleomycin, adriamycin, cyclophosphamide, vincristine, and prednisone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85:417–422, 1976.
2. SWEET DL, GOLOMB HM, ULTMANN JE, ET AL. Cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine (COMLA) combination sequential chemotherapy for advanced diffuse histiocytic lymphoma. Ann Intern Med 92:785–790, 1980.
3. TODD M, CADMAN E, SPIRO P, ET AL. A follow-up of a randomized study comparing two chemotherapy treatments for advanced diffuse histiocytic lymphoma. J Clin Oncol 1:986–993, 1984.
4. SKARIN AT, CANELLOS GP, ROSENTHAL DS, ET AL. Improved prognosis of diffuse histiocytic and undifferentiated lymphoma by use of high dose methotrexate alternating with standard agents (M-BACOD). J Clin Oncol 1:91–98, 1983.
5. FISHER RI, DEVITA VT, JR, HUBBARD SM, ET AL. Diffuse aggressive lymphomas: increased survival after alternating flexible sequences of ProMACE and MOPP chemotherapy. Ann Intern Med 98:304–309, 1983.
6. KLIMO P, and CONNORS JM. MACOP-B chemotherapy for the treatment of diffuse large-cell lymphoma. Ann Intern Med 102: 596–602, 1985.
7. FISHER RI, HUBBARD SM, DEVITA VT, ET AL. Factors predicting long-term survival in diffuse mixed, histiocytic, or undifferentiated lymphoma. Blood 58:45–50, 1981.
8. HANDE KR, FISHER RI, DEVITA VT, ET AL. Diffuse histiocytic lymphoma involving the gastrointestinal tract. Cancer 41:1984–1989, 1978.
9. ROSEN PJ, FEINSTEIN DI, PATTENGALE PK, ET AL. Convoluted lymphocytic lymphomas in adults: a clinicopathologic entity. Ann Intern Med 89:319–325, 1978.
10. NATHWANI BN, DIAMOND LW, WINBERG CD, ET AL. Lymphoblastic lymphoma: a clinicopathologic study of 95 patients. Cancer 48:2347–2357, 1981.
11. ZIEGLER JL, DEISSEROTH AB, APPLEBAUM FR, ET AL. Burkitt's lymphoma—a model for intensive chemotherapy. Semin Oncol 4:317–323, 1977.
12. LUKES RJ, and COLLINS RD. Immunologic characterization of human malignant lymphoma. Cancer 34:1488–1503, 1974.
13. GREER JP, YORK JC, COUSAR JB, ET AL. Peripheral T-cell lymphoma: a clinicopathologic study of 42 cases. J Clin Oncol 2:788–798, 1984.
14. OVIATT DL, COUSAR JB, FLEXNER JM, ET AL. Malignant lymphoma of follicular center cell origin in humans: small transformed (noncleaved) cell lymphoma of the non-Burkitt's type. Cancer 52: 1196–1201, 1983.
15. LEVINE AM, TAYLOR CR, SCHNEIDER DR, ET AL. Immunoblastic sarcoma of T-cell versus B-cell origin: clinical features. Blood 58:52–61, 1981.
16. ZIEGLER JL. Burkitt's lymphoma. N Engl J Med 305:735–745, 1981.
17. MAGRATH IT, JANUS C, EDWARDS BK, ET AL. An effective therapy for both undifferentiated (including Burkitt's) lymphomas and lymphoblastic lymphomas in children and young adults. Blood 63: 1102–1111, 1984.
18. SULLIVAN MP, and RAMIREZ I. Curability of Burkitt's lymphoma with high-dose cyclophosphamide—high-dose methotrexate therapy and intrathecal chemoprophylaxis. J Clin Oncol 3:627–636, 1985.
19. COLEMAN CN, COHEN JR, BURKE JS, ET AL. Lymphoblastic lymphoma in adults: results of a pilot protocol. Blood 57:679–684, 1981.
20. LEVINE AM, FORMAN SJ, MEYER PR, ET AL. Successful therapy of convoluted T-lymphoblastic lymphoma in the adult. Blood 61: 92–98, 1983.
21. WEINSTEIN HJ, CASSADY JR, and LEVEY R. Long-term results of the APO protocol (vincristine, doxorubicin [adriamycin], and prednisone) for treatment of mediastinal lymphoblastic lymphoma. J Clin Oncol 1:537–540, 1983.
22. PHILLIPS GL, HERZIG RH, LAZARUS HM, ET AL. Treatment of resistant malignant lymphoma with cyclophosphamide, total body irradiation, and transplantation of cryopreserved autologous marrow. N Engl J Med 310:1557–1561, 1984.
23. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
24. SWEET DL, COLLINS RD, STEIN RS, ET AL. Prognostic significance of the Lukes and Collins classification in patients treated with COMLA. Cancer Treat Rep 66:1107–1111, 1982.
25. NEWCOMER LN, NERENBERG MI, CADMAN EC, ET AL. The usefulness of the Lukes-Collins classification in identifying subsets of diffuse histiocytic lymphoma responsive to chemotherapy. Cancer 50:439–443, 1982.
26. COLEMAN M. Chemotherapy for large-cell lymphoma: optimism and caution. Ann Intern Med 103:140–142, 1985.

Differential Drug Sensitivity of Human Neuroblastoma Cells[1,2]

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A 6-day in vitro growth-inhibition assay was used to determine relative sensitivity of six human neuroblastoma cell lines to three classes of cancer chemotherapeutic agents: antimetabolites (methotrexate, methasquin, and cytarabine); antimitotics (vincristine, vinblastine, vindesine, colchicine, and demecolcine); and antibiotics (dactinomycin and doxorubicin). Human fibroblast lines served as a reference standard. Whereas response to antimetabolites by four of five neuroblastoma lines tested was similar to that of fibroblasts, SK-N-MC cells were significantly more sensitive (fivefold to 16-fold) to the three drugs. Human neuroblastoma cell lines were also differentially sensitive to antimitotics, especially to vincristine. In particular, SK-N-MC, IMR-32, and LA-N-2 cells were threefold to 17-fold more sensitive to this drug than were the SK-N-SH and SK-N-BE(2) lines. With the exception of SK-N-SH, all of the human neuroblastoma lines were considerably more sensitive to vincristine than were human fibroblasts. The same three highly vincristine-responsive lines were also significantly more sensitive to the other alkaloidal drugs as compared to fibroblasts. However, human neuroblastoma and fibroblastic cells responded similarly to the two antibiotics. The only cellular attribute consistently correlated with greater sensitivity to the antimitotics among the six cell lines tested was expression of the neurotransmitter biosynthetic enzyme choline acetyltransferase. A differential response to the vinca alkaloids was also exhibited by clonal sublines of SK-N-SH. One subpopulation (SH-EP) expressing a nonneuronal, substrate-adherent, variant phenotype was at least fourfold to eightfold more drug sensitive than its neuroblastic counterpart (SH-SY5Y) or the parental SK-N-SH population. In addition to confirming reports of clinical efficacy of the various classes of agents, these data have demonstrated a variation in response possibly related to type or degree of neuronal differentiation and emphasize the importance of understanding the phenotypic diversity commonly observed in neuroblastoma. [Cancer Treat Rep 70:959–965, 1986]

While neuroblastoma is one of the most common solid tumors of early childhood, the prognosis for patients with this disease remains poor. The tumor is responsive to many chemotherapeutic agents and to radiation therapy; however, the survival rate of patients > 1 year of age with advanced, stage IV disease is generally $< 10\%$ (1,2). Thus, it is clear that much more needs to be known about neuroblastoma and its response to therapeutic drugs for an effective treatment to be developed.

One recent approach to this problem has been to evaluate the activities of chemotherapeutic agents against human neuroblastoma cells in vitro. Although the in vitro growth inhibition of tumor cells by drug does not always correlate with sensitivity in vivo (3), recent studies have shown a good correlation between the drug sensitivities of neuroblastoma in patients and those of derived cell lines when grown in vitro or in nude mice (4–6). Moreover, the use of an in vitro assay of tumor cell response to cytotoxic agents has several advantages. First, it allows rapid screening of multiple agents which can subsequently be tested in vivo. Second, availability of a substantial number of well-characterized human neuroblastoma cell lines (or subpopulations of a single cell line) derived from different patients and with differing morphological or biochemical properties (7–12) permits assessment of chemotherapeutic activity against a wide spectrum of tumor cells

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representative of this malignancy. This latter aspect is very important with respect to neuroblastoma because of the extensive heterogeneity of cell types, in terms of neuronal cell properties, that exist within this one disease.

The present study was undertaken to assess the sensitivities of six human neuroblastoma cell lines to three different classes of chemotherapeutic agents: (*a*) antimetabolites (methotrexate, methasquin, and cytarabine), (*b*) antimitotics (vincristine, vinblastine, vindesine, colchicine, and demecolcine), and (*c*) antibiotics (dactinomycin and doxorubicin). In addition, we have carried out growth-inhibition studies of morphologically and biochemically distinct subpopulations of cells which arise spontaneously within the human neuroblastoma cell line SK-N-SH (8,13,14) to assess possible differential drug sensitivities among neuroblastoma cell variants.

MATERIALS AND METHODS

Materials

Medium and antibiotics were purchased from Grand Island Biological Co, NY; fetal bovine serum was from Microbiological Associates, Walkersville, MD. Dactinomycin (Cosmegen) was obtained from Merck Sharp and Dohme, Inc, Rahway, NJ; doxorubicin (Adriamycin) from Farmitalia, Milan, Italy; vinblastine (Velban) and desacetyl vinblastine amide (vindesine) from Eli Lilly and Co, Indianapolis, IN; colchicine from Fisher Scientific Co, Springfield, NJ; demecolcine (Colcemid) from Ciba Pharmaceutical Products, Inc, Summit, NJ; and cytarabine (Cytosar) from The Upjohn Co, Kalamazoo, MI. Methotrexate, vincristine (Oncovin), and methasquin were generous gifts from Lederle Laboratories, Pearl River, NY, Eli Lilly and Co, and Dr. Harry B. Wood of the Cancer Chemotherapy National Service Center, respectively.

Cell Culture

All of the human fibroblast and neuroblastoma cell lines and clones have been described (7-14). Clinical data relating to the six neuroblastoma lines are summarized in table 1. Four of the lines [SK-N-BE(2), SK-N-SH, SK-N-MC, and IMR-32] as well as the clones derived from SK-N-SH have near-diploid chromosome numbers (13,14). Routine karyotype analyses carried out through the course of this and other (12,14) studies have shown that the cell lines are karyotypically homogeneous and stable. The LA-N-1 and LA-N-2 lines are heteroploid (10,13) and are somewhat chromosomally variable. The cloned lines were tested for drug sensitivity 4–5 months after isolation. Cells were grown in 25-cm^2 flasks at 37°C in a 1:1 mixture of Eagle's minimum essential medium and Ham's F-12 nutrient medium containing 15% heat-inactivated fetal bovine serum, 100 IU of penicillin/ml and 100 μg of streptomycin/ml. Neurotransmitter biosynthetic enzyme activities and growth characteristics of cell lines and clones are summarized in table 2.

Assay of Drug Sensitivity

For determination of drug sensitivity, a growth-inhibition assay system (15) was modified slightly for the slowly growing human cells. Briefly, 25-cm^2 flasks were seeded with 5×10^5 cells. Duplicate cultures were incubated with various concentrations of drug (usually four to six graded concentrations per assay) or without drug for 6 days. Cultures were then rinsed with phosphate-buffered saline (PBS), subjected to 0.12% trypsin and to 0.02% EDTA in PBS, and triturated to yield a single-cell suspension. The total cell number per flask was determined by means of a Coulter model F counter. Drug sensitivity was calculated from the average number of cells in treated cultures compared with untreated cultures at each drug concentration. The dose effective in inhibiting growth of and/or killing 50% of the cell population by the end of the 6-day culture period, ie, the ED_{50}, was obtained from a line fitted to these data. The ED_{50} for each drug/cell line combination was determined from at least three separate assays, unless otherwise indicated. Statistical significance of drug response for neuroblastoma cell lines compared with fibroblastic cell lines as controls was determined using Student's t-test.

RESULTS

Antimetabolites

To determine the sensitivity of human neuroblastoma cell lines to the toxic effects of antimetabolic drugs such

TABLE 1.—Neuroblastoma cell lines: clinical data

Cell line	Patient age (yrs)	Tissue source	Prior therapy*	Response to therapy	Reference No.
SK-N-BE(2)	2	Bone marrow	RT; VCR, CTX, ADR, ACT	None	11
SK-N-SH	4	Bone marrow	RT; VCR, CTX, DNR, FMdU, ADR	None	8
SK-N-MC	14	Retro-orbital tumor	RT; VCR, CTX, ADR, ACT	None or minimal	8
IMR-32	1	Primary tumor	None		7
LA-N-1	2	Bone marrow	CTX, DTIC, VCR, ADR	None	10
LA-N-2	3	Primary tumor	None		10

*RT = radiotherapy (specimens were not obtained from within the radiation port); VCR = vincristine; CTX = cyclophosphamide; ADR = doxorubicin; ACT = dactinomycin; DNR = daunorubicin; FMdU = trifluoromethyl-2′-deoxyuridine; and DTIC = dacarbazine.

TABLE 2.—Characteristics of human neuroblastoma cell lines and clones

Cell line	Population doubling time (hrs)	Saturation density (cells/cm^2 × 10^4)	Neurotransmitter enzyme activity*		
			TH	DBH	CAT
SK-N-BE(2)	34	147	54	5.8	ND
SK-N-SH	46	136	0.017	15	ND†
SH-SY5Y	54	200	0.007	11	ND
SH-IN	21	57	0.056	11	ND
SH-EP	44	7.5	ND	ND	ND
SK-N-MC	22	106	ND	ND	3.5
IMR-32			1.5	ND	0.55
LA-N-1	58	107	3.1	ND	ND†
LA-N-2	87	76	0.23	3.7	9.4

*nmols/hr/mg of protein. TH = tyrosine hydroxylase; DBH = dopamine-β-hydroxylase; and CAT = choline acetyltransferase. ND = not detectable.

†A more sensitive assay measured low or barely detectable levels of specific CAT activity in LA-N-1 and SK-N-SH cells (12).

as methotrexate, its synthetic analog methasquin, and cytarabine, dose-response curves were generated and the ED_{50} was determined for five human neuroblastoma cell lines and three human fibroblast controls. As shown in table 3, the response of three of the five tumor cell lines to the drugs was within the range of that of the fibroblasts. SK-N-MC cells were significantly more sensitive to methotrexate, methasquin, and cytarabine (16.2-, 5.0-, and 12.4-fold, respectively). IMR-32 was slightly more sensitive to methotrexate when compared to fibroblast controls. As illustrated in figure 1a, the dose-response curves for the antimetabolite-sensitive SK-N-MC line were parallel to those obtained for other lines.

Microtubule-Disruptive Agents

To determine the degree of sensitivity of human neuroblastoma cell lines to the toxic effects of microtubule-disrupting drugs, ED_{50} values were obtained for vinca alkaloids (vincristine, vinblastine, and its derivative vindesine) and colchicum alkaloids (colchicine and demecolcine) with six human neuroblastoma cell lines and compared to those with four human fibroblast lines (table 4). Although there was a differential response to this group of agents, dose-response curves were linear over a fivefold to tenfold range in concentration and, except for LA-N-1 cells, were generally parallel, as illustrated in figure 1b. The slope of curves obtained with LA-N-1 cells was consistently shallower, possibly reflecting the phenotypic variability exhibited by these heteroploid cells (unpublished data). Sensitivity of SK-N-BE(2) and SK-N-SH cells to the five agents resembled that of controls. By contrast, SK-N-MC and IMR-32 cells were highly sensitive to this class of drugs. The greatest sensitivity was to vincristine, but both lines exhibited moderate sensitivity to vindesine, vinblastine, and colchicine, and marginal, albeit statistically significant, sensitivity to demecolcine. LA-N-2 cells were also highly sensitive to vincristine and vinblastine, the only

TABLE 3.—Response of human neuroblastoma and fibroblast cell lines to antimetabolites

Cell line	Methotrexate		Methasquin		Cytarabine	
	ED_{50} ± SD*	Relative sensitivity	ED_{50} ± SD*	Relative sensitivity	ED_{50} ± SD*	Relative sensitivity
Fibroblast						
F-ECH	14 ± 3(3)				38 ± 31(4)	
F-LSO	14 ± 2(2)		12 ± 5(3)		4 ± 1(2)	
WI-38	35 ± 6(3)					
Mean	21 ± 12(3)	1	12 (1)	1	21 ± 24(2)	1
Neuroblastoma						
SK-N-BE(2)	15 ± 2(2)	1.4	8 ± 1(2)	1.5	6.4 ± 1.8(3)	3.3
SK-N-SH	29 ± 7(3)	0.7	29 ± 6(2)	0.4	3.8 ± 0.1(4)	5.5
SK-N-MC	1.3 ± 1.0(2)	16.2†	2.4 ± 0.3(3)	5.0†	1.7 ± 0.1(2)	12.4†
IMR-32	8.9 ± 1.2(4)	2.4†			7.1 ± 3.0(2)	3.0
LA-N-1	20 ± 2(2)	1.0	18 (1)	0.7		

*Values = ng/ml. No. of individual assays is shown in parentheses.

†$P < 0.01$ when compared to fibroblasts as control.

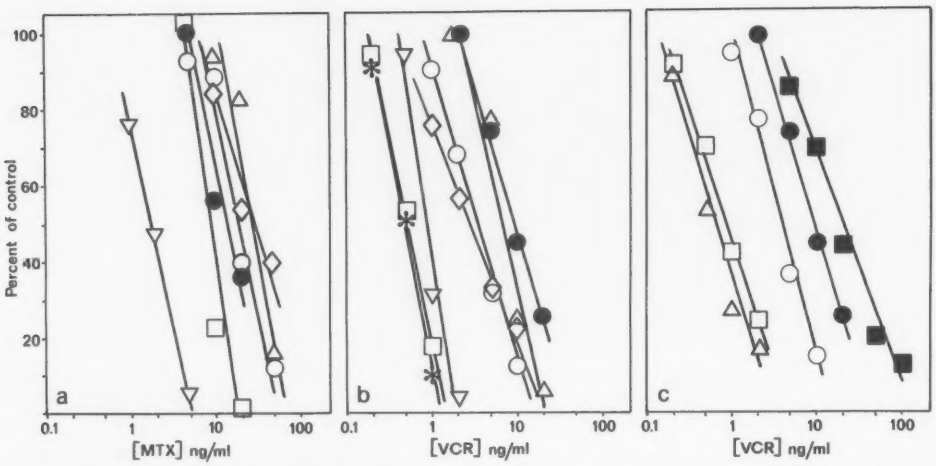

FIGURE 1.—Representative dose-response curves for human neuroblastoma cells exposed to methotrexate or vincristine. a. Response to methotrexate by SK-N-MC (▽), IMR-32 (□), LA-N-1 (◇), SK-N-BE(2) (○), SK-N-SH (△) and fibroblastic F-ECH (●) cell lines. b. Response to vincristine by LA-N-2 (✱) and the 6 lines listed in a. c. Response to vincristine by 3 morphological variant clones of SK-N-SH [flattened SH-EP (△), intermediate SH-IN (□), and neuroblastic SH-SY5Y (○) cells] and fibroblast controls (●, ■).

two antimitotics tested, whereas LA-N-1 cells exhibited moderate sensitivity to the former and marginal sensitivity to the latter agent.

Dactinomycin and Doxorubicin

Dose response data with drugs which intercalate into DNA were obtained for six human neuroblastoma cell lines and compared with data obtained for four fibroblast lines (table 5). None of the neuroblastoma cell lines examined showed a greater sensitivity to either dactinomycin or doxorubicin nor were they any less responsive, with the possible exception of LA-N-2.

Differential Sensitivity of Human Neuroblastoma Cell Variants

To examine human neuroblastoma cells with different morphological and biochemical phenotypes, ED_{50} and relative sensitivity were determined for clones of the human neuroblastoma cell line SK-N-SH (fig 1c and table 6). This cell line comprises two predominant types of cells (8,14), one expressing morphological and biochemical characteristics of neuroblasts (represented by SH-SY5Y) and the other having a flattened, epithelial-like morphology and no detectable neurotransmitter biosynthesizing enzyme activity (represented by

TABLE 4.—Response of human neuroblastoma and fibroblast cell lines to alkaloidal drugs

	Vincristine		Vinblastine		Vindesine		Colchicine		Demecolcine	
Cell line	$ED_{50} \pm SD$*	Relative sensitivity	$ED_{50} \pm SD$*	Relative sensitivity	$ED_{50} \pm SD$*	Relative sensitivity	$ED_{50} \pm SD$*	Relative sensitivity	$ED_{50} \pm SD$*	Relative sensitivity
Fibroblast										
WI-38	9.6 ± 2.4(2)									
F-ECH	8.8 ± 0.6(2)		2.3 ± 0.3(2)		3.2 ± 1.6(2)		5.1 ± 0.1(2)		11 ± 1.0(2)	
F-LSO	18 ± 1(2)		8.9 ± 0.7(2)				15 ± 6.3(4)		11 ± 1.0(2)	
F-ALF	3.4 ± 0.7(3)		2.0 ± 0.3(3)		3.5 ± 0.8(3)		4.1 ± 0.1(3)		9.3 ± 1.0(3)	
Mean	10 ± 6.0(4)	1	4.4 ± 3.9(3)	1	3.4 ± 0.2(2)	1	8.1 ± 6.0(3)	1	10.4 ± 1.0(3)	1
Neuroblastoma										
SK-N-BE(2)	3.0 ± 1.1(4)	3.3	4.0 ± 1.0(3)	1.1	4.8 ± 1.4(4)	0.7	3.0 ± 0.7(2)	2.7	6.7 ± 0.3(3)	1.6‡
SK-N-SH	6.3 ± 1.0(4)	1.6	2.5 ± 0.7(4)	1.8	8.3 ± 1.6(3)	0.4	4.0 ± 0.6(4)	2.0	6.6 ± 0.1(3)	1.6‡
SK-N-MC	0.88 ± 0.07(3)	11†	0.93 ± 0.07(3)	4.7‡	0.64 ± 0.12(3)	5.3†	2.2 ± 0.5(4)	3.7‡	4.6 ± 1.3(3)	2.3‡
IMR-32	0.40 ± 0.13(4)	25†	0.88 ± 0.15(3)	5.0‡	0.47 ± 0.20(4)	7.2†	1.4 ± 0.2(3)	5.8‡	4.8 ± 0.7(3)	2.2‡
LA-N-1	1.8 ± 0.5(3)	5.6‡	1.7 ± 0.1(2)	2.6						
LA-N-2	0.47 ± 0.03(2)	21†	0.41 ± 0.12(2)	10.7†						

*Values = ng/ml. No. of individual assays performed is shown in parentheses.
†$P < 0.001$ when compared to fibroblasts as control.
‡$P < 0.01$ when compared to fibroblasts as control.

TABLE 5.—Response of human neuroblastoma and fibroblast cell lines to antibiotics

Cell line	Dactinomycin		Doxorubicin	
	$ED_{50} \pm SD$*	Relative sensitivity	$ED_{50} \pm SD$*	Relative sensitivity
Fibroblast				
WI-38	0.49 ± 0.06(2)			
F-ECH	0.26 ± 0.03(2)		9.1 ± 0.9(2)	
F-LSO			6.2 ± 0.8(2)	
F-ALF	0.18 ± 0.02(3)		7.3 ± 1.6(3)	
Mean	0.31 ± 0.16(3)	1	7.5 ± 1.5(3)	1
Neuroblastoma				
SK-N-BE(2)	0.62 ± 0.13(3)	0.50	11.0 ± 3.9(3)	0.68
SK-N-SH	0.51 ± 0.05(4)	0.61	6.3 ± 0.95(3)	1.2
SK-N-MC	0.46 ± 0.12(3)	0.67	8.3 ± 1.0(3)	0.90
IMR-32	0.21 ± 0.03(4)	1.48	3.8 ± 1.0(3)	2.0
LA-N-1	0.68 ± 0.12(2)	0.46	5.7 ± 0.6(2)	1.3
LA-N-2	1.1 ± 0.1(2)	0.28†		

*Values = ng/ml. No. of individual assays is shown in parentheses.
†$P < 0.01$ when compared to fibroblasts as control.

SH-EP). SH-IN has characteristics intermediate between these two cell types (14). While the neuroblast clone showed a drug response similar to that of the parental SK-N-SH cell line with respect to the toxic actions of all drugs tested, the SH-EP variant was significantly more sensitive to both antimetabolite and antimitotic drugs (table 6). A greater sensitivity to the cytoskeletal-disrupting drugs was seen also for SH-IN cell variants.

DISCUSSION

The present study sought to disclose possible differences in drug response of six well-established human neuroblastoma cell lines and of different subpopulations of cells represented within the same parental cell line to three classes of chemotherapeutic agents. The cytotoxicity assay used in these studies was chosen for several reasons. First, this method is rapid and highly reproducible, enabling the simultaneous screening of many drugs at different concentrations for many cell lines. Second, while this assay procedure may be less sensitive statistically than colony-forming assays, we sought to identify only large differences in drug response among the human neuroblastoma cell lines. Moreover, drug sensitivity of mass cultures, where cell-cell interaction can affect response to drug, may better represent the situation existing during cancer therapy. Therefore, the growth-inhibition assay proved to be the assay of choice. In these studies, fibroblasts or fibroblast-like cells were used as reference standards to obtain values from which to assess relative drug sensitivity

TABLE 6.—Differential response to drugs by human neuroblastoma SK-N-SH cell clones with differing cellular phenotypes*

Drug	Fibroblast controls	SK-N-SH	Clones		
			SH-SY5Y	SH-IN	SH-EP
Antimetabolites					
Methotrexate	21 ± 12(3)	29 ± 7(3)	53 ± 1(2)		11 ± 2(2)
Methasquin	12 (1)	29 ± 6(2)	20 ± 1(2)		1.4(1)
Cytarabine	21 ± 24(2)	3.8 ± 0.1(4)	3.1 ± 0.2(2)		
Antimitotics					
Vincristine	10.0 ± 6.0(4)	6.3 ± 1.0(4)	3.9 ± 1.3(4)	0.77 ± 0.03(2)†[a,b]	0.92 ± 0.56(5)†[a,b]
Vinblastine	4.4 ± 3.9(3)	2.5 ± 0.7(4)	2.2 ± 0.3(2)	0.75 ± 0.09(2)†[a,b]	0.54 ± 0.09(3)†[a,b]
Vindesine	3.4 ± 0.2(2)	8.3 ± 1.6(3)	5.4 ± 0.5(3)		0.65 ± 0.13(3)†[a,b]
Colchicine	8.1 ± 6.0(3)	4.0 ± 0.6(4)	3.2 ± 0.3(3)		2.0 ± 0.06(3)†[a,b]
Demecolcine	10.4 ± 1.0(3)	6.6 ± 0.1(3)†[a]	6.8 ± 0.5(3)†[a]		4.2 ± 1.3(4)†[a,b]
Antibiotics					
Dactinomycin	0.31 ± 0.16(3)	0.51 ± 0.05(4)	0.70 ± 0.06(3)	0.21 ± 0.01(2)	0.24 ± 0.01(3)
Doxorubicin	7.5 ± 1.5(3)	6.3 ± 1.0(3)	5.9 ± 0.5(3)		3.2 ± 0.9(3)

*Values = mean $ED_{50} \pm SD$ (ng/ml). No. of individual assays is shown in parentheses.
†$P < 0.001$: [a] compared to fibroblasts; [b] compared to neuroblastic lines SK-N-SH and SH-SY5Y.

and to test for statistical significance when comparing the different human neuroblastoma cell lines.

Differential Drug Sensitivities Among Different Human Neuroblastoma Cell Lines

The importance of the present findings is not the demonstration of drug sensitivity by human neuroblastoma cell lines in vitro, as has been shown by others (5,6), but rather the manifestation of differential sensitivity among the six different neuroblastoma cell lines tested. There was a differential response to the inhibitory effects of both antimitotic and antimetabolite drugs, especially vincristine. Whereas SK-N-BE(2) and SK-N-SH cell lines showed no greater sensitivity to either class of drugs when compared to fibroblast controls, the four other cell lines showed a significantly greater response to several of the drugs. Vinca alkaloids and, where tested, colchicum alkaloids showed a greater growth-inhibitory action against the SK-N-MC, IMR-32, LA-N-2, and, to a lesser extent, the LA-N-1 cell lines when compared to the other two neuroblastoma cell lines or fibroblast controls. The findings confirm and extend previous in vivo and in vitro studies on the selective toxicity of these drugs for human neuroblastoma cells (5,6). With respect to the growth-inhibitory actions of the antimetabolite drugs, SK-N-MC cells were relatively the most sensitive, showing a fivefold to 16-fold greater sensitivity to the three agents than did four other neuroblastoma cell lines and fibroblast controls. In contrast, responses of all six neuroblastoma lines and controls to dactinomycin and doxorubicin were similar, with less than fourfold differences in relative sensitivity. This finding is of interest with respect to observations that these drugs have limited effectiveness in the treatment of advanced disease (16,17).

These observations provide a basis on which to explain and evaluate the differential response of human neuroblastoma in vivo to antitumor drugs. Comparison of the morphological, growth, biochemical, and karyotype characteristics of the six neuroblastoma cell lines showed no apparent correlation between cell morphology, doubling time, saturation density, or type or degree of chromosomal abnormality and drug response (table 2). One consistent correlation, however, was seen between drug sensitivity and the neurotransmitter phenotype, viz, choline acetyltransferase (CAT) activity. SK-N-MC, IMR-32, LA-N-2, and, to a lesser extent, LA-N-1 contain CAT activity, whereas SK-N-BE(2) and SK-N-SH have no or barely detectable levels of this enzyme (12); all but SK-N-MC also contain activities for norepinephrine biosynthetic enzymes (table 2). Those lines with CAT activity are significantly more sensitive to the antimitotic agents tested than are SK-N-BE(2) and SK-N-SH. The relationship between neurotransmitter expression and drug sensitivity requires further study but suggests that the functional and/or developmental state of the neuroblastoma cell can influence its responsiveness to antitumor drugs.

Differential Drug Sensitivity Among Different Cell Types Within the Same Neuroblastoma Cell Line

Numerous studies have shown the existence of subpopulations of cells in animal and human primary tumors, selected variants, and spontaneous metastases with differential sensitivity to various chemotherapeutic drugs (3,18). In the present study, a similar differential response to chemotherapeutic drugs is shown for cloned human neuroblastoma cell lines with diverse phenotypes. The flat cell variants of SK-N-SH show a fourfold to eightfold greater sensitivity to the vinca alkaloids when compared to neuroblastic cells derived from the same cell line. Moreover, the atypical slope of the LA-N-1 dose response curve with vincristine (fig 1b) may reflect a similar, as yet undetermined, difference in sensitivity between the two morphological subpopulations of this line (unpublished data). Whether the differential effects observed are related to the substrate-adherent properties of the variant cells or to specific intracellular metabolic alterations associated with the phenotypic interconversion that these cells are known to undergo (14) remains to be determined. Further study of differential drug response of the phenotypically variant cells arising in human neuroblastoma cell populations in vitro (8,14,19-21) and of the natural phenotypic heterogeneity of human neuroblastoma cells in vivo is important to the development of effective treatment of this childhood cancer.

REFERENCES

1. SIMONE JV. The treatment of neuroblastoma. J Clin Oncol 2:717-718, 1984.
2. ROSEN EM, CASSADY JR, FRANTZ CN, ET AL. Neuroblastoma: the Joint Center for Radiation Therapy/Dana-Farber Cancer Institute/Children's Hospital experience. J Clin Oncol 2:719-732, 1984.
3. HEPPNER GH, DEXTER DL, DENUCCI T, ET AL. Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res 38:3758-3763, 1978.
4. HELSON L, DAS SK, and HAJDU SI. Human neuroblastoma in nude mice. Cancer Res 35:2594-2599, 1975.
5. SIEGEL MM, CHUNG HS, RUCKER N, ET AL. In vitro and in vivo preclinical chemotherapy studies of human neuroblastoma. Cancer Treat Rep 64:975-979, 1980.
6. HILL BT, and WHELAN RD. Assessments of the sensitivities of cultured human neuroblastoma cells to anti-tumor drugs. Pediatr Res 15:1117-1122, 1981.
7. TUMILOWICZ JJ, NICHOLS WW, CHOLON JJ, ET AL. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res 30:2110-2118, 1970.
8. BIEDLER JL, HELSON L, and SPENGLER BA. Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. Cancer Res 33:2643-2652, 1973.
9. SCHLESINGER HR, GERSON JM, MOORHEAD PS, ET AL. Establishment and characterization of human neuroblastoma cell lines. Cancer Res 36:3094-3100, 1976.
10. SEEGER RC, RAYNER SA, BANERJEE A, ET AL. Morphology, growth, chromosomal pattern, and fibrinolytic activity of two new human neuroblastoma cell lines. Cancer Res 37:1364-1371, 1977.
11. BIEDLER JL, ROFFLER-TARLOV S, SCHACHNER M, ET AL. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res 38:3751-3757, 1978.
12. ROSS RA, BIEDLER JL, SPENGLER BA, ET AL. Neurotransmitter-synthesizing enzymes in 14 neuroblastoma cell lines. Cell Mol Neurobiol 1:301-312, 1981.

13. BIEDLER JL, MEYERS MB, and SPENGLER BA. Homogeneously staining regions and double minute chromosomes, prevalent cytogenetic abnormalities of human neuroblastoma cells. Adv Cell Neurobiol 4:268–307, 1983.
14. ROSS RA, SPENGLER BA, and BIEDLER JL. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. JNCI 71:741–747, 1983.
15. BIEDLER JL, and RIEHM H. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic and cytogenetic studies. Cancer Res 30:1174–1184, 1970.
16. TAN C, ROSEN G, GHAVIMI F, ET AL. Adriamycin (NSC-123127) in pediatric malignancies. Cancer Chemother Rep Part 3, vol 6(2): 259–266, 1975.
17. EVANS AE. Treatment of neuroblastoma. Cancer 30:1595–1599, 1972.
18. TSURUO T, and FIDLER IJ. Differences in drug sensitivity among tumor cells from parental tumors, selected variants, and spontaneous metastases. Cancer Res 41:3058–3064, 1981.
19. BERNAL S, THOMPSON RA, GILBERT F, ET AL. In vitro and in vivo growth characteristics of two different cell populations in an established line of human neuroblastoma. Cancer Res 43:1256–1260, 1983.
20. BIEDLER JL, ROSS RA, MEYERS MB, ET AL. Transdifferentiation capability of human neuroblastoma cells in culture. Proc Am Assoc Cancer Res 25:41, 1984.
21. ROSS RA, and BIEDLER JL. Presence and regulation of tyrosinase activity in human neuroblastoma cell variants in vitro. Cancer Res 45:1628–1632, 1985.

Phase II Evaluation of Aclarubicin in Refractory Adult Acute Leukemia: A Southwest Oncology Group Study[1,2]

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Aclarubicin, a new anthracycline antibiotic, was used to treat 24 adult patients with refractory adult leukemia, using a total dose of 300 mg/m² (75 mg/m²/day × 4). There were 20 patients with acute myelogenous and four with acute lymphoblastic leukemia. Approximately two-thirds of the patients had a Karnofsky score of ≤ 2, and two-thirds had received two or more previous induction programs. Interim bone marrow evaluation was obtained in 18 of 30 remission induction courses and revealed marked hypocellularity in 14, inadequate specimens in three, and persistent disease in one. Seven patients received more than one course. Two patients refused further therapy. In patients with myelogenous leukemia, there were two complete remissions lasting 10 and 16 months and one partial remission lasting 4½ months. There were no responders in patients with lymphoblastic leukemia. Toxicity included profound leukopenia and thrombocytopenia, moderate nausea and vomiting, diarrhea, and mucositis. There were no cardiac symptoms associated with the drug infusion, but there were three late events possibly associated with anthracycline cardiotoxicity. Used in this dosage schedule, aclarubicin is an active, but toxic, agent in the acute myelogenous leukemias. [Cancer Treat Rep 70:967-969, 1986]

The long-term administration of anthracycline antibiotics is limited by severe cardiomyopathy, which is directly related to the cumulative dose (1). The finding of a new anthracycline, aclarubicin, which is active in refractory acute leukemia with purportedly much less cardiotoxicity than currently available compounds, prompted us to evaluate this agent in a group study (2).

Data from early reports suggested that the appropriate dose was 300 mg/m² (3,4). Our experience with aclarubicin at this dose in patients with refractory acute leukemia forms the basis of this report.

PATIENTS AND METHODS

Patient Eligibility

Patients ≥ 16 years of age with a pathologically verified diagnosis of acute leukemia and an absolute marrow infiltrate of ≥ 50% leukemic cells, who had either relapsed from or were refractory to one or more combination chemotherapeutic regimens, were candidates for this protocol. Patients had to have recovered from nadir cell counts of their previous treatment, a Karnofsky performance status of > 30%, and a serum creatinine and serum bilirubin of ≤ 2.0 mg/dl. Patients who had a prior cumulative dose of daunorubicin > 400 mg/m² or doxorubicin > 300 mg/m² were eligible if cardiac function measured by nuclear scanning was normal. Patients with a past history of congestive heart failure or ischemic heart disease were excluded, but patients with only cardiac arrhythmia were permitted entrance at the discretion of the primary investigator. They could receive no concomitant chemotherapy.

Prestudy evaluation required medical history and physical examination with notation of the performance status. Laboratory parameters included a cbc with differential and platelet count, bone marrow aspirate or biopsy, urinalysis, serum BUN and creatinine, blood glucose, serum bilirubin, SGOT, alkaline phosphatase, chest x-ray, ECG, echocardiogram, or cardiac nuclear scan. The bone marrow was to be repeated on Day 10 of each course and prior to each treatment.

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Treatment Protocol

Aclarubicin was given in a dose of 75 mg/m^2 daily for 4 days for a total dose of 300 mg/m^2 every 3 weeks. It was infused in 100 ml of 5% glucose in water over 15 minutes. If there was a < 50% reduction in the marrow blast infiltrate by Day 21 after the second course, patients were removed from the study. If the infiltrate was reduced by > 50%, two subsequent induction courses could be given. Patients achieving a complete remission were to receive three courses of consolidation therapy, consisting of 75 mg/m^2 of aclarubicin daily for 3 days for a total dose of 225 mg/m^2 per course, administered at 21-day intervals or at bone marrow recovery. No maintenance was used.

Assessment of Response

Complete remission was defined as follows: bone marrow with blasts plus progranulocytes < 10%, lymphocytes < 20%, and normal-appearing trilinear maturation, both qualitatively and quantitatively except for drug-induced morphologic changes. The peripheral blood prerequisites were hemoglobin > 11.0 g/dl, granulocytes > 1000/μl, absence of blasts, and platelet count > 100,000/μl. Patients had to be free of symptoms, with no evidence of extramedullary leukemic infiltration on physical examination.

TABLE 1.—Patient characteristics*

Characteristic	ALL	AML
Total	4	20
Males/females	3/1	11/9
Median age in yrs (range)	34 (19–75)	50 (19–67)
Preleukemia	0	1
Therapy-related leukemia	0	1
Previous chemotherapy		
No. of induction programs		
1	1	7
2	1	11
≥ 3	2	2
SWOG performance status†		
≤ 2	2	13
3	0	4
Unknown	2	3
Disease status		
Failure to obtain 1st remission	0	9
1st relapse	2	1
Failure to obtain 2nd remission	1	7
Relapse after transplant	1	0
2nd relapse	0	2
3rd relapse	0	1

*Unless otherwise specified, values = No. of patients. ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia.

†SWOG (Southwest Oncology Group) criteria: 0 = 90%–100%, 1 = 70%–80%, 2 = 50%–60%, and 3 = 30%–40% (Karnofsky rating).

RESULTS

Twenty-five patients were registered on this protocol. One patient was ineligible due to lack of a bone marrow specimen. Patient characteristics are summarized in table 1. Most of the patients entered the study with a good performance status. Twenty patients had acute myelogenous leukemia, with all French-American-British cell types being represented including progranulocytic and erythroleukemia. One patient had antecedent acquired sideroblastic anemia and one had undergone therapy for lymphoma. Nine patients (45%) had failed to obtain a first remission; one (5%) was in relapse; seven (35%) failed to obtain a second remission; two (10%) were in second relapse; and one (5%) was in third relapse. Thirteen patients had more than one remission induction program, most often doxorubicin, 6-thioguanine, cytarabine, vincristine, and prednisone for the first, and high-dose cytarabine for the second. One patient with acute myelogenous leukemia died on Day 9 and could not be evaluated for response to therapy but is included in the toxicity data. Seven of the patients had two or more courses. Two patients refused further therapy. There were two complete remissions (11%) lasting 8 and 16 months, and one partial remission (5%) lasting 4½ months.

The four patients with acute lymphocytic leukemia [three men and one woman, with a median age of 34 years (range, 19–75)] were more diverse in terms of age spectrum and previous chemotherapy. They included one patient who had had bone marrow transplantation. After the first of the four proposed infusions of the first course, one patient with acute lymphocytic leukemia refused further therapy and was unevaluable; the three other patients failed to respond to aclarubicin.

There were interim bone marrow evaluations performed during 18 of 30 remission induction courses. Fourteen of 18 (77.8%) demonstrated hypocellularity, three of 18 (16.8%) were technically inadequate, and one specimen (5.5%), obtained on Day 17, revealed persistent disease. The median time until recovery or relapse occurred was 27 days (range, 18–45).

Toxic Effects

The infusion of aclarubicin resulted in nausea and vomiting in approximately one-half of the patients and diarrhea in one-third (table 2). Mucositis of variable degree also occurred in one-half of the patients. The most serious problem was the profundity of the myelosuppression: thrombocytopenia > leukopenia > anemia. All of the patients required platelet transfusions and iv antibiotics for both bacterial and fungal infections or infiltrates on chest x-ray.

There were no cardiac symptoms associated with the drug infusions. However, there were three late events that may have been related to anthracycline cardiotoxicity. Five days after the last dose, one patient had ventricular tachycardia, which was treated successfully. A second patient had nonspecific ST-T abnormalities in the second week after treatment. The third patient had atrial flutter-fibrillation associated with congestive

TABLE 2.—Toxic effects

Toxic effect	No. of patients	
	ALL ($N = 3$)	AML ($N = 20$)
Nausea/vomiting ≥ 2*	2	11
Diarrhea	1	6
Mucositis	2	10
Leukopenia (< 500 granulocytes/mm^3)	3	19
Thrombocytopenia (< 49,999 platelets/mm^3)	3	19
Anemia (≤ 8.9 g/dl)	3	18
Cardiotoxicity	0	3†
Creatinine elevation	0	3‡
Bilirubin elevation	1	3§

*Actual vomiting which can be either prevented by therapy, or occurring < 6 times/day, or worse.

†1 patient had ventricular tachycardia 5 days after the last drug dose; 1 had ST-T ECG abnormalities 14 days after the last drug dose; and 1 had congestive heart failure in conjunction with pulmonary infiltrate, fever, hypotension, and atrial flutter-fibrillation 9 days after the last drug dose.

‡In patients who were receiving aminoglycosides, cephalosporins, and amphotericin.

§2 patients during 2nd induction in conjunction with sepsis.

heart failure, which the attending physicians attributed to sepsis in the setting of fever, pulmonary infiltrate, and hypotension occurring 9 days after the last anthracycline dose.

One patient had both renal and hepatic failure associated with sepsis and progressive disease. Two patients receiving the second remission induction course had transient hyperbilirubinemia without concomitant enzyme changes. Two other patients had an increase in alkaline phosphatase. There were no sustained kidney or liver problems except for the one patient alluded to above. Whether the difficulties were drug-related is unclear. Most of the deaths were attributable to infection and most occurred during the period of marrow hypoplasia. Infections were also the cause of death when patients refused further therapy and the disease was progressive.

DISCUSSION

The response rates for complete remission induction (11%) and toxic effects obtained in this group study are very similar to those reported by Warrell et al (4) (13%) from a single-institution study. These investigators, after determining that 100 mg/m^2 of aclarubicin on each of 2 days failed to produce marrow hypoplasia, treated their patients for 3 days or 300 mg/m^2/course. They found no advantage in escalating the dose to a total of 360 mg/m^2/course. They, too, found responses in patients previously treated with daunorubicin and doxorubicin, but also failed to produce remission in patients with refractory acute lymphocytic leukemia. A Danish trial in patients refractory to daunorubicin, which used daily 1-hour iv infusions at a dose of 80 mg/m^2 for 3 days, followed by the same dose daily for 2 days after 2–3 weeks, failed to produce the same marrow hypoplasia that we noted, but resulted in a complete remission rate of 20.5% in eight patients (5).

The original Japanese (6) and French (3,7) studies were carried out with a small dose (10–30 mg/m^2/day) until remission was obtained, and these investigators also recommended a total dose of 300 mg/m^2 for remission induction.

This trial confirms that aclarubicin has activity in acute nonlymphocytic leukemia, even in patients refractory to doxorubicin or daunorubicin. The agent had no apparent utility in three patients with refractory acute lymphocytic leukemia.

REFERENCES

1. LENAZ L, and PAGE JA. Cardiotoxicity of adriamycin and related anthracyclines. Cancer Treat Rev 3:111–120, 1976.
2. DANTCHEV D, SLIOUSSARTCHOUK V, PAINTRAND M, ET AL. Electron microscopic studies of the heart and light microscopic studies of the skin after treatment of golden hamsters with adriamycin, detorubicin, AD-32, and aclacinomycin. Cancer Treat Rep 63:875–888, 1979.
3. MATHE G, GIL M-A, DELGADO M, ET AL. Phase II trial of aclacinomycin in acute leukemia and lymphosarcoma. Recent Results Cancer Res 74:217–222, 1980.
4. WARRELL RP, JR, ARLIN ZA, KEMPIN SJ, ET AL. Phase I–II evaluation of a new anthracycline antibiotic, aclacinomycin A, in adults with refractory leukemia. Cancer Treat Rep 66:1619–1623, 1982.
5. PEDERSON-BJERGAARD J, BRINCKER H, ELLEGARD J, ET AL. Phase II trial of aclacinomycin A in acute non-lymphocytic leukemia refractory to treatment with daunorubicin. Proc ASCO 25:208, 1984.
6. YAMADA K, NAKAMURA T, TSURUO T, ET AL. A phase II study of aclacinomycin A in acute leukemia in adults. Cancer Treat Rev 7:177–182, 1980.
7. DE JAGER R, DELGADO M, BAYSSAS M, ET AL. Phase II study of aclacinomycin A (ACM) in acute leukemia and leukemic lymphosarcoma. Proc Am Assoc Cancer Res and ASCO 22:171, 1981.

Pharmacokinetics of the Thymidylate Synthase Inhibitor N^{10}-Propargyl-5,8-dideazafolic Acid (CB3717) in the Mouse[1,2]

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The tissue distribution, excretion, and metabolism of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717) have been investigated in the mouse. Following 100 mg/kg of 2-^{14}C-CB3717 ip, levels of radioactivity in the brain, testes, muscle, heart, and lung equilibrated slowly with those in the plasma and were no longer significantly lower 5 hours (lung) and 12 hours (brain, testes, muscle, and heart) after administration. In contrast, concentrations of ^{14}C in the liver and kidney were markedly higher than those in the plasma at all time points studied (1.3 hours–23 days). High-performance liquid chromatographic (HPLC) analysis of livers removed 5 hours after drug administration and kidneys excised 24 hours after treatment indicated that, at these time points, > 50% of the radioactivity was in the form of unchanged CB3717. Furthermore, HPLC analysis of plasma removed over the period 0.5–6 hours demonstrated that all of the ^{14}C could be accounted for as CB3717. Although the accumulation and retention of radioactivity in the liver and kidney were also apparent following 20 and 200 mg/kg of ^{14}C-CB3717, the effect was less marked at the lower dose, thereby suggesting dose-dependent pharmacokinetics. In excretion studies (0–48 hours), the major route of elimination was found to be via the feces, with 46% of the ^{14}C recovered; 26% of the dose was recovered as unchanged CB3717. Radioactivity excreted in the urine accounted for 20% of the administered ^{14}C, while CB3717 eliminated via this route represented 15% of the dose. In addition to CB3717, a metabolite was detected in the feces which comprised 8% of the dose administered. The metabolite was shown to be 4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoic acid (CB3751) by HPLC and mass spectrometry. The formation of CB3751 could be catalyzed in vitro by the contents of the cecum and prevented in vivo by antibiotic pretreatment and is therefore considered to be the result of bacterial metabolism. CB3717 binds extensively to plasma proteins (92%; concentration range, 25–250 μM). These studies have shown that CB3717 does not apparently undergo extensive host metabolism in vivo, and therefore the biological properties of this novel antimetabolite are probably a function of the parent compound. In addition, the accumulation of CB3717 in the liver and kidney may be related to the hepatotoxic and nephrotoxic effects of this drug. [Cancer Treat Rep 70:971–979, 1986]

N^{10}-Propargyl-5,8-dideazafolic acid (CB3717, fig 1a) is a tight-binding inhibitor of the enzyme thymidylate synthase (EC 2.1.1.45), the terminal enzyme in the de novo pathway for the biosynthesis of thymidine monophosphate (1). CB3717 is a competitive inhibitor of thymidylate synthase, with respect to 5,10-methylene tetrahydrofolate, with a Ki of approximately 5 nM (2,3). It is cytotoxic to cells in culture, producing 50% inhibition of growth in a variety of cell lines at concentrations generally within the range of 1–10 μM (1,3–5). Measurements of dihydrofolate and nucleotide pools, in conjunction with studies showing that cytotoxicity can be reversed by thymidine alone, have clearly demonstrated that the cytotoxicity of CB3717 in vitro is mediated primarily through inhibition of thymidylate synthase (3).

When tested in vivo against murine tumors, CB3717

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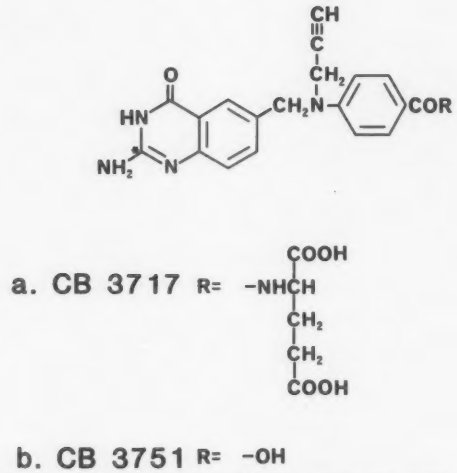

FIGURE 1.—Structures of CB3717 (N^{10}-propargyl-5,8-dideazafolic acid) (a) and CB3751 [4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoic acid] (b). *Indicates the position of the ^{14}C label.

displays only modest activity, with the exception of one particular L1210 leukemia model in which CB3717 has curative activity (1,6). In toxicity studies performed in mice, CB3717 induced transient elevations in plasma alanine transaminase levels, suggesting liver toxicity. Testicular atrophy was also observed. However, the dose-limiting side effect was renal damage, as demonstrated by histopathology and serum biochemistry.[6]

On the basis of its potent and specific biochemical properties, coupled with the activity of this novel antimetabolite in vitro and in vivo, CB3717 was selected for clinical study. In a phase I trial the dose-limiting toxic effect of CB3717 in man was found to be renal damage, as made evident by reductions in glomerular filtration rates. However, hepatic toxicity, ie, elevations in plasma alanine transaminase levels, was the most frequent side effect, occurring in > 50% of the patients (7). During the phase I trial of CB3717, responses were observed in patients with a variety of tumor types (7). These promising early results stimulated phase II studies which are currently under way. Activity in breast and ovarian cancer has already been confirmed (A. H. Calvert and D. L. Alison, personal communication).

The pharmacokinetic studies described herein were performed in conjunction with the preclinical toxicology study of CB3717 in an attempt to explain the observed toxic effects by describing the tissue distribution, metabolism, and excretion of the drug. The dose of CB3717 employed in most of these studies (100 mg/kg) and the route of administration (ip) were chosen on the basis of toxicity and antitumor studies as a maximum tolerated dose, on repeat administration, which produces a large percentage of cures in the CB3717-sensitive L1210 leukemia model (8).

MATERIALS AND METHODS

Nonradioactive CB3717 (fig 1a) as the diacid was a gift from ICI Pharmaceuticals (Alderly Park, Macclesfield, Cheshire, England). The synthesis of the desglutamyl metabolite of CB3717, 4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoic acid (CB3751, fig 1b) will be described elsewhere. Streptomycin and benzylpenicillin were purchased from Sigma (Poole, Dorset, England). 2-^{14}C-CB3717 (fig 1a) was prepared as a custom synthesis by Amersham International (Amersham, Bucks, England) and supplied at 12.5 mCi/mmol with a radiochemical purity of > 96% as determined by thin-layer chromatography. Prior to use, ^{14}C-CB3717 was dissolved in nonradioactive solutions of CB3717 in 0.15 M $NaHCO_3$ (final pH, 9–9.5). The chemical and radiochemical purity of the injection solutions was then confirmed by high-performance liquid chromatography (HPLC). ^{14}C-hexadecane (Amersham, Bucks, England) was used to determine the efficiencies of both sample combustion and scintillation counting. Unless otherwise stated, all other chemicals were analytical grade and obtained from standard suppliers.

Animal Studies

These were performed using male ($C57BL \times DBA_2)F_1$ mice (body weight, 27–33 g) supplied by the Institute of Cancer Research (London, England). Animals were allowed free access to tap water and food in all experiments; they were housed in wire-bottomed cages, with particular care being taken to remove all fecal pellets, thereby reducing the possibility of coprophagy. For excretion studies, mice were placed individually in glass metabolism cages (Jencons, Leighton Buzzard, Beds, England) and urine and feces were collected at room temperature over two successive 24-hour periods. Dose solutions of CB3717 were prepared fresh on each occasion and administered ip at 10 ml/kg within 2 hours. The final specific activities of the injection solutions were as follows: for tissue distribution and excretion studies at 100 mg/kg of ^{14}C-CB3717—2.3–3.4 mCi/mmol, following 20 mg/kg of ^{14}C-CB3717—12.5 mCi/mmol, and after 200 mg/kg of ^{14}C-CB3717—1.1 mCi/mmol; for comparison of plasma CB3717 and ^{14}C levels following 100 mg/kg of ^{14}C-CB3717—10.8 mCi/mmol.

Mice requiring antibiotic pretreatment received benzylpenicillin (0.5 g/L) in the drinking water for 5 days prior to the experiment; fresh solutions were prepared each day. In addition, streptomycin was given orally at doses of 50 mg/kg (2.5 mg/ml in water) 5 days and 1 day prior to the experiment.

[6] Jackman AL, Calvert AH, and Newell DR. Unpublished results.

Tissue Distribution Studies

Animals were killed by exsanguination following direct cardiac puncture under diethyl ether anesthesia at various times after drug administration. The kidneys, liver, heart, testes, and brain were excised and samples of lung and muscle from the upper leg were also removed. Tissues were rinsed and blotted dry, and the wet weight was recorded. Pieces of liver and brain (100–400 mg) and the entire sample for all other tissues were wrapped in 5.5-cm discs of Whatman No. 1 filter paper and placed in a Combustocone and combusted within 48 hours (storage at −20°C). Heparinized whole blood samples (20 IU/ml, 0.1 ml) were placed on Combustopads and then placed in Combustocones. Similarly, 0.1 ml of plasma was taken following centrifugation of whole blood at 2000 × *g* for 2 minutes at room temperature. Sample combustion was performed over a period of 1 minute using a Packard Instruments Model 306 oxidizer, with 0.25 ml of Combustaid being added immediately prior to combustion. $^{14}CO_2$ generated was collected in 9 ml of Carbo-sorb and scintillation counting performed on a model SL40 Intertechnique liquid scintillation spectrometer (Kontron Intertechnique, St. Albans, Herts, England) following the addition of 9 ml of Permafluor V scintillant. All materials used in sample combustion were supplied by Packard Instruments (Reading, Berks, England).

Excretion Studies and Plasma ^{14}C Analysis for Comparison With HPLC

Scintillation counting was performed on samples (0.02–0.1 ml) digested with 1 ml of NCS solubilizer (Hopkin and Williams, Romford, Essex, England). Following digestion, samples were neutralized (0.05 ml of glacial acetic acid) and analyzed by scintillation counting in 10 ml of cocktail T scintillant (BDH, Poole, Dorset, England). Plasma and urine were added directly to the solubilizer and feces were analyzed following homogenization (5% wt/vol) in 0.1 M tris (hydroxymethyl)ammonia-HCl buffer (pH 10.0) using a Teflon-glass homogenizer.

HPLC Analyses

These were performed on a Waters Associates liquid chromatograph (Waters Associates, Northwich, Cheshire, England) operating in isocratic mode. Samples were analyzed on a 25 × 0.46-cm μBondapack C18 column (Waters Associates) fitted with a 5 × 0.21-cm Whatman ODS precolumn (Whatman, Maidstone, Kent, England) with a mobile phase consisting of methanol/water/glacial acetic acid (35/64/1, vol/vol) flowing at 2 ml/minute. The effluent of the column was monitored at 254 nm and 280 nm and the output of the detectors recorded on an automatic integrator (Trivector, Sandy, Beds, England). Peaks were identified by retention volume and 254 nm/280 nm absorbance ratio and quantitated by peak area at 280 nm using external standards. Prior to HPLC analysis, samples (0.1–0.3 ml) of plasma; urine; fecal, liver, or kidney homogenates; and cecal contents incubates were mixed vigorously with 2 volumes of methanol and centrifuged at 2000 × *g* for 15 minutes at 4°C. From 0.01 to 0.05 ml of the methanolic supernatant was analyzed by HPLC. Kidney and liver homogenates were prepared as described for fecal homogenates. The assay was validated by the addition of known amounts of CB3717 to control mouse plasma and urine to give concentrations of 2–400 μM. Linearity in both cases was satisfactory ($r > 0.999$) and recovery from plasma high (93.2% ± 2.3%, $\bar{x} \pm SE$). Recovery from kidney, liver, or fecal homogenates and cecal contents incubates was taken as 100% and the values obtained may therefore represent an underestimate of the actual drug levels. For the analysis of ^{14}C in the effluent of the HPLC, 1-ml fractions were collected and counted in 10 ml of cocktail T scintillant.

Metabolism by Cecal Contents

Ceca from three untreated or three antibiotic-pretreated mice were removed and placed in 50 ml of 0.15 M sodium phosphate-buffered NaCl (pH 7.4). The contents of the ceca were washed out and the ceca were removed. ^{14}C-CB3717 (5.4 μCi) was added to give a final concentration of 400 μM. Samples were incubated under aerobic conditions at 37°C and 1-ml samples removed at 0 time and at intervals thereafter. Aliquots of the samples were analyzed by HPLC as described above.

The efficacy of antibiotic pretreatment in eliminating the gut flora was demonstrated by subjecting the incubate to microbiological analysis at the beginning and at the end of the experiment. These studies were performed by Dr. W. Simpson (Institute of Cancer Research, Chalfont St Giles, Bucks, England). At the beginning of the incubation, no organism could be isolated from the cecal contents incubate prepared from antibiotic-pretreated mice. However, by the end of the experiment a heavy growth of *Flavobacterium meningosepticum*, a penicillin-resistant organism, was observed. Similarly, fecal pellets from the antibiotic-pretreated mice used in in vivo experiments contained only small numbers of *F. meningosepticum* and coagulase-negative staphylococci. In contrast, feces collected from mice prior to antibiotic treatment contained a normal flora, ie, *Bacteroides* spp, lactobacilli, coliforms, fecal streptococci, *Proteus mirabilis*, coagulase-negative staphylococci, and *Pseudomonas maltophilia*.

Mass Spectral Analysis

The CB3717 metabolite (CB3751, fig 1b) observed in cecal contents incubates and in feces from mice not pretreated with antibiotics was isolated prior to mass spectral analysis. The feces used were those collected during the period 24–48 hours following the administration of 100 mg/kg of ^{14}C-CB3717. The cecal contents incubate (30 ml) or fecal homogenate (34 ml) was mixed with an equal volume of 3.2 M tetrabutylammonium

hydroxide (pH 10.0) for 15 minutes. The samples were then extracted with an equal volume (ie, twice the original volume) of butan-1-ol by mixing vigorously for 15 minutes using a magnetic stirrer, and the two layers separated by centrifugation (550 × *g*, 4°C, 15 minutes). The upper organic layer was decanted, the aqueous phase was re-extracted with an additional equal volume of butan-1-ol, and the pooled extracts were concentrated to dryness by rotary evaporation under reduced pressure. The concentrate was solubilized in 12 ml of 0.18 M acetic acid, and 2-ml aliquots were applied to each of six C18 Sep-pak cartridges (Waters Associates). These were each eluted successively with 4 ml of 0.18 M acetic acid, 4 ml of 0.18 M acetic acid/methanol (1/1 vol/vol), and 4 ml of methanol. The 0.18 M acetic acid/methanol (1/1 vol/vol) fractions were pooled and concentrated by rotary evaporation. CB3751 present in the concentrate was isolated by HPLC with fraction collection. Following removal of the HPLC solvent by rotary evaporation, the purified metabolite was dissolved in 0.1 ml of dimethyl sulfoxide which had previously been dried over activated 3A sieves and distilled from calcium hydride at 58°C/3 mm Hg. The metabolite solution, or 100 ng of synthetic CB3751 dissolved in 0.1 ml of dimethyl sulfoxide, was derivatized by permethylation (9) using a 1 M solution of sodium methyl sulfinyl methide in dimethyl sulfoxide prepared at 70°C, as described by Durst (10).

Electron impact mass spectra were recorded using a VG 7070H spectrometer and analyzed using a VG 2235 data system (VG, Manchester, England). The ionizing voltage was 70 eV, trap current was 100 μA, and ion-source temperature was 200°C. Samples were introduced on the direct insertion probe.

Protein Binding

The protein binding of CB3717 (25–250 μM) to heparinized plasma (10 IU/ml) prepared from untreated mice was determined by HPLC following separation of the free drug by using the Centrifree micropartition system (Amicon, Stonehouse, Glos, England). CB3717 solutions (50 μl) were added to 0.95-ml aliquots of plasma and the ultrafiltrate was obtained by centrifugation at 2000 × *g* for 20 minutes at 15°C. Aliquots (20 μl) of the ultrafiltrate were analyzed by HPLC, and protein binding was determined by comparison with CB3717 solutions in 0.15 M $NaHCO_3$ treated in an identical manner. Loss of CB3717 on the ultrafiltration membrane was < 5% over the concentration range studied. Statistical differences between samples were demonstrated by using Student's t-test.

RESULTS

Tissue Distribution

The tissue levels of ^{14}C over the time period 1.3–48 hours following the administration of 100 mg/kg of ^{14}C-CB3717 are given in table 1. Peak plasma ^{14}C levels were observed at the first time point (1.3 hours), decaying thereafter. Over the entire time course, most of the radioactivity present in whole blood was found in the plasma, indicating that partition into the erythrocytes was limited. Of the tissues sampled, levels of ^{14}C in the brain, testes, muscle, and heart were not significantly different from those in the plasma at 12 hours or thereafter ($P > 0.1$). In lung, however, levels of radioactivity were higher than those in plasma at 24 and 48 hours ($P = 0.03$ and 0.06, respectively). The most notable observation was that at all time points studied, levels of ^{14}C in both liver and kidneys greatly exceeded those in plasma (greater than tenfold). Furthermore, radioactivity was cleared less rapidly from these organs than from plasma such that at 48 hours, levels in the liver were approximately 100-fold those in plasma, and levels in the kidneys were 1000 times greater. Thus, as shown in figure 2, during the period 1.3–48 hours ^{14}C present

TABLE 1.—Tissue distribution of radioactivity in the mouse following 100 mg/kg of ^{14}C-CB3717 ip: 1.3–48 hours*

	Time (hrs)				
Tissue	1.3	5	12	24	48
Plasma	130 ± 10	75 ± 38	12 ± 8.4	2.2 ± 0.3	1.2 ± 0.3
Blood	69 ± 3	52 ± 27	5.5 ± 0.5	1.2 ± 0.1	0.8 ± 0.1
Kidney	680 ± 260	1600 ± 960	1000 ± 860	1300 ± 380	1300 ± 440
Liver	2200 ± 490	750 ± 290	220 ± 20	130 ± 24	77 ± 15
Lung	50 ± 10	35 ± 22	22 ± 8.7	16 ± 10	14 ± 12
Heart	21 ± 4	14 ± 7	7.2 ± 4.5	6.6 ± 8.4	2.9 ± 2.2
Muscle	16 ± 5	11 ± 2	9.0 ± 5.4	1.7 ± 0.6	2.9 ± 1.8
Testes	43 ± 14	12 ± 6	8.1 ± 0.7	2.3 ± 0.8	3.8 ± 2.3
Brain	5.2 ± 2.5	3.7 ± 1.3	3.9 ± 2.8	0.7 ± 0.5	1.2 ± 0.6

*Values are the ^{14}C concentrations in drug equivalents expressed as nmol/g wet weight or, for plasma and blood, nmols/ml ± SD of determinations performed on 5 mice at each time point.

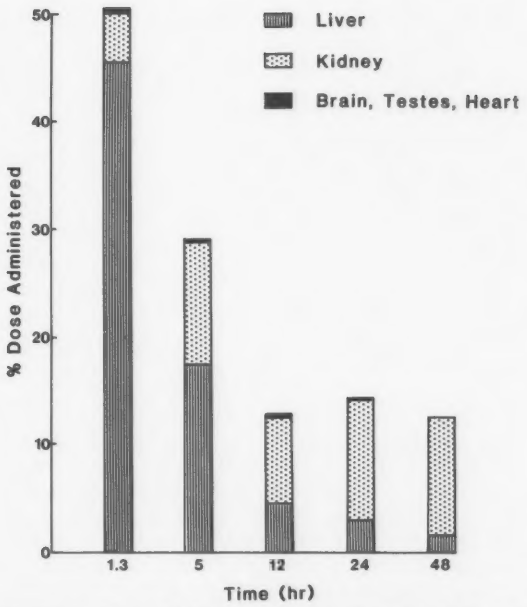

FIGURE 2.—Tissue distribution of ^{14}C over the time period 1.3–48 hours following ip administration of 100 mg/kg of 2-^{14}C-CB3717. Data are given as % of dose administered present in the organs at each time point. For concentrations and SD, *see* table 1.

in the kidneys and liver accounted for a large percentage of the dose administered, while the other organs that were removed intact contributed little.

In view of the high levels of ^{14}C observed in the liver and kidneys, an extended distribution study was performed over the period 1–23 days during which sampling was restricted to the plasma, liver, and kidneys. In addition to the dose used in the short-term experiment, 20 mg/kg and 200 mg/kg of ^{14}C-CB3717 were also studied.

TABLE 2.—Tissue distribution of radioactivity in the mouse following 20, 100, and 200 mg/kg of ^{14}C-CB3717 ip: 1–23 days*

Time (days)	Dose (mg/kg)	Plasma (nmols/ml)	Kidney (nmols/g)	Liver (nmols/g)
1	20	< 0.5	5.8 ± 0.5	9.1 ± 1.8
	100	2.4 ± 0.6	2000 ± 340	170 ± 20
	200	17.4 ± 4.6	5600 ± 560	590 ± 70
3	20	< 0.5	3.5 ± 1.6	2.8 ± 1.2
	100	0.8 ± 0.1	1500 ± 660	50 ± 14
	200	1.7 ± 0.2	3500 ± 970	170 ± 80
6	100	< 0.5	1100 ± 660	16 ± 4
10	20	< 0.5	0.9 ± 0.1	1.2 ± 0.2
	100	< 0.5	930 ± 200	6.3 ± 0.5
15	100	< 0.5	780 ± 250	5.3 ± 1.9
23	100	< 0.5	690 ± 100	2.5 ± 0.6
	200	< 0.5	1600 ± 200	8.4 ± 1.8

*Values are the ^{14}C concentration in drug equivalents and are the mean ± SD of determinations in 3 mice at each dose at each time point.

As shown in table 2, levels of radioactivity in both the liver and kidneys were greatly in excess of those in plasma following 100 and 200 mg/kg of ^{14}C-CB3717. Following 20 mg/kg of ^{14}C-CB3717, radioactivity in plasma was below the limit of detection (< 0.5 μM) at all of the time points. A comparison of ^{14}C levels in the kidneys following 100 and 200 mg/kg of ^{14}C-CB3717 indicates that there is an approximate dose relationship. However, kidney ^{14}C levels following 20 mg/kg of ^{14}C-CB3717 were far lower than would be predicted from the levels observed at the two higher doses. Thus, dose dependency is apparent in the renal retention of radioactivity following ^{14}C-CB3717. Similarly, at earlier time points (< 10 days), levels of ^{14}C in the liver are not linearly dose-related, since retention in the liver is more pronounced with increasing dose.

In an attempt to identify metabolites of CB3717 in the plasma of mice treated with 100 mg/kg of ^{14}C-CB3717, levels of both parent compound (determined by

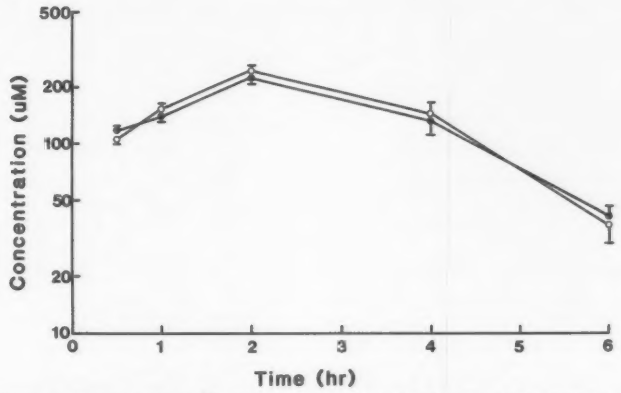

FIGURE 3.—Plasma levels of ^{14}C (•) and CB3717 (○) following ip administration of 100 mg/kg of 2-^{14}C-CB3717. Points are the means and bars the SE of determinations made in 5 mice at each time point.

TABLE 3.—Urinary and fecal excretion of ^{14}C, CB3717, and CB3751 in the mouse following 100 mg/kg of ^{14}C-CB3717 ip*

	Normal mice			Antibiotic-treated mice
	Time (hrs)			Time (hrs)
	0–24	24–48	0–48	0–48
Fecal ^{14}C	34 ± 6	12 ± 3	46 ± 13	67 ± 6
Fecal CB3717	22 ± 4	4 ± 2	26 ± 3	41 ± 3
Fecal CB3751	2 ± 1	6 ± 2	8 ± 1	< 1
Urinary ^{14}C	14 ± 1	5 ± 1	20 ± 1	18 ± 2
Urinary CB3717	11 ± 1	4 ± 1	15 ± 1	14 ± 1

*Values are % of dose administered excreted in the form given. Data are the mean ± SE of observations from 6 mice.

HPLC) and total radioactivity were compared over the time period 0.5–6 hours. As shown in figure 3, during this period all of the radioactivity in the plasma could be accounted for as intact CB3717. In a separate experiment, mice were treated with 100 mg/kg of CB3717, and after 5 hours the livers were removed, homogenized, and analyzed for intact CB3717 using HPLC. In this study, 11% ± 1% ($\bar{x}$ ± SE, $n = 3$) of the dose could be accounted for as unchanged CB3717 in the liver. Similarly, kidneys from mice treated with 100 mg/kg of CB3717 were removed 24 hours after administration and found to contain 6.5% ± 1.1% ($\bar{x}$ ± SE, $n = 4$) of the dose as unchanged CB3717. Values (% dose administered) for ^{14}C present in the liver and kidneys at these time points were 15% and 11%, respectively (data derived from table 1). This indicates that at least 70% of the ^{14}C present in the liver at 5 hours and 58% of the ^{14}C present in the kidneys at 24 hours were in the form of the parent compound.

Excretion

The urinary and fecal excretion of radioactivity and intact CB3717 was examined following the administration of 100 mg/kg of ^{14}C-CB3717. During these studies a metabolite was detected in feces which was chromatographically indistinguishable (retention vol and 254 nm/280 nm absorbance ratio) from authentic CB3751 (fig 1b). The structure of this metabolite was confirmed by mass spectrometry. Table 3 gives the levels of ^{14}C, CB3717, and CB3751 excreted in the feces and urine during the period 0–48 hours. Within this time, most of the administered radioactivity was excreted, predominantly via the fecal route. Most of the ^{14}C in the feces could be accounted for as CB3717 and CB3751, with CB3717 accounting for the major part, particularly during the first 24 hours. Of the ^{14}C present in the urine, the majority was again in the form of CB3717; however, CB3751 was not detected, ie, < 0.5% of the dose excreted in the urine within 48 hours was CB3751.

In view of the data concerning methotrexate (11), an experiment was performed using mice that had been pretreated with antibiotics in order to determine the role of the gut bacteria in the metabolism of CB3717 to CB3751. As shown in table 3, pretreatment with antibiotics led to a significant increase in the percent of the dose excreted as CB3717 in the feces ($P = 0.003$) and a reduction in CB3751 excretion to below the limit of detection (< 1% of the dose within 24 hours). In other respects, the excretion of ^{14}C and CB3717 was not significantly altered.

Metabolism and Metabolite Identification

To further investigate the metabolism of CB3717, the ceca of three mice were excised and the contents of the ceca were removed and incubated in the presence of 400 μM ^{14}C-CB3717. A parallel experiment was performed using the ceca from mice pretreated with antibiotics. As shown in figure 4, the cecal contents of normal mice readily effected the conversion of CB3717 to CB3751. In contrast, the formation of CB3751 was greatly reduced when the cecal contents of antibiotic-pretreated mice were used.

To allow mass spectral analysis of the metabolite identified as CB3751 following both the in vivo and in vitro metabolism of CB3717, aliquots of the fecal homogenate and cecal contents incubate were extracted with butan-1-ol and fractionated on Sep-pak C18 cartridges. Table 4 shows the recovery of ^{14}C at each stage for feces and cecal contents incubates. Thus, in both cases overall recovery of radioactivity was > 80% with the 0.18 M acetic acid/methanol (1/1 vol/vol) fraction containing the most radioactivity. For both fecal and cecal contents samples, HPLC analysis of this fraction indicated only two radioactive compounds, namely CB3717 and CB3751, with retention volumes > 10 ml. More than 80% of the applied radioactivity was associated with these two components.

The peak with the appropriate retention volume for CB3751 was isolated in each case from the 0.18 M acetic acid/methanol (1/1 vol/vol) Sep-pak fraction by HPLC and subjected to mass spectrometry following permethylation. Figure 5 shows the mass spectrum of the metabolite isolated from the cecal contents incubate.

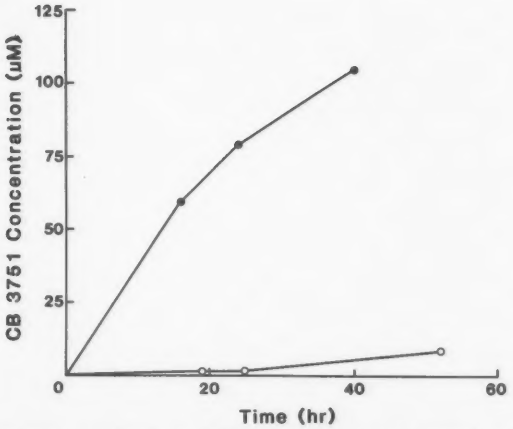

FIGURE 4.—Formation of CB3751 during the in vitro incubation of CB3717 (400 μM) with cecal contents removed from normal mice (●) or mice pretreated with antibiotics (○). Data are the means of duplicate estimates performed at each time point.

This spectrum was identical to that given by authentic CB3751 and the metabolite isolated from feces. Thus, CB3751 (fig 1b) has been unequivocally identified as an in vivo metabolite of CB3717. The ability of the cecal contents to catalyze the formation of this metabolite in vitro and the inhibitory effect of antibiotic pretreatment strongly suggest that CB3751 is formed as a product of bacterial metabolism.

TABLE 4.—Recovery of radioactivity during the fractionation of mouse fecal homogenates and in vitro cecal contents incubates*

Sample	Fecal homogenate	Cecal contents incubate
Homogenate/incubate	100	100
Butanol extract	100	92
Sep-pak fraction		
Acetic acid	28	18
Acetic acid/methanol	43	65
Methanol	10	12

*Values are % of radioactivity present in initial homogenate or cecal contents incubate. *See* text for fractionation procedure. Data are the mean of duplicate determinations performed on each fraction.

Protein Binding

The protein binding of CB3717 was studied over the concentration range 25–250 μM and found to be linear ($r = 0.973$) at 92% ± 2% ($\bar{x} \pm SD$, $n = 8$). Thus, CB3717 binds extensively to plasma proteins.

DISCUSSION

The pharmacokinetic studies reported herein have described the in vivo fate of ^{14}C and CB3717 following the administration of 2-^{14}C-CB3717.

In tissue distribution studies the most notable observation was that the liver and kidneys accumulate and retain levels of ^{14}C greatly in excess of those found in the plasma and other tissues. Both in mice[6] and in patients (7), hepatic and renal toxic effects have been

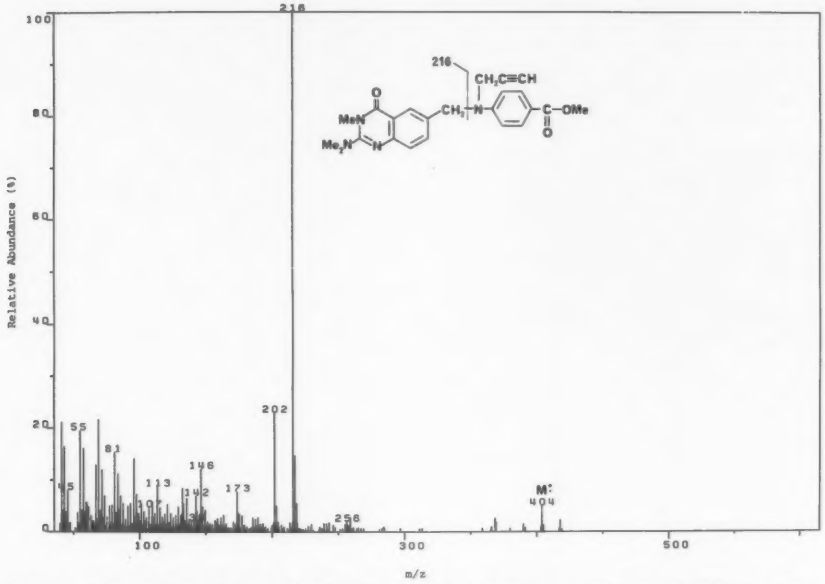

FIGURE 5.—Mass spectrum of permethylated metabolite isolated following the incubation of CB3717 (400 μM) with cecal contents of normal mice. *See* text for the isolation procedure, derivatization, and mass spectrometry conditions.

observed which may, therefore, be related to the extensive exposure of these two organs to the drug. Although the precise mechanisms of toxicity are as yet unidentified, preliminary experiments in mice suggest that renal toxicity is due to drug precipitation in the nephron during acidification of the urine (12). Thus, CB3717 solubility in urine at pH 7, 8, and 9 is 0.04, 0.4, and 4 mg/ml, respectively (D. R. Newell, unpublished results); in one patient, 5% of the administered dose was found as unchanged CB3717 in the kidneys 8 days after treatment (13). In this respect, CB3717 is apparently similar to methotrexate (14). Thus, alkalinization regimens, as used during high-dose methotrexate therapy, should readily overcome CB3717 renal toxicity.

One interesting observation that emerged from studies on the long-term tissue distribution of CB3717 is an apparent dose dependence. This suggests that clinical evaluation of low-dose CB3717 might be worthwhile in an attempt to reduce hepatic and renal side effects.

In mice, administered ^{14}C was eliminated primarily in the feces, with most of the radioactivity excreted as the parent compound. Significant quantities of ^{14}C were also excreted in the urine, again mainly as CB3717. In addition to the parent compound, one CB3717 metabolite was isolated from feces and shown to be CB3751, the desglutamyl metabolite. The formation of this metabolite could be catalyzed in vitro by the cecal contents of normal mice, an activity which was greatly reduced when the cecal contents of mice pretreated with antibiotics were utilized. Similarly, CB3751 could not be detected following CB3717 administration in the feces of mice pretreated with antibiotics. These observations strongly suggest that CB3751 is formed in vivo by the bacterial metabolism of CB3717 within the gastrointestinal tract. Again, CB3717 is analogous to methotrexate in that 4-amino-4-deoxy-N^{10}-methylpteroic acid has been described as a metabolite of methotrexate in mice (15) and man (16,17). However, whereas 4-amino-4-deoxy-N^{10}-methylpteroic acid can be detected in urine and plasma, CB3751 was only recovered from feces in the present study. Thus, CB3751 is unlikely to contribute to either the systemic toxicity or the antitumor activity of CB3717. In any case, relative to CB3717, CB3751 is a weak inhibitor of thymidylate synthase (approximately 100-fold worse) and not significantly cytotoxic in vitro [concentration to give 50% inhibition of L1210 cell growth > 200 μM (A. L. Jackman, unpublished results)]. Thus, the metabolism of CB3717 to CB3751 represents inactivation.

The evidence presented in this study argues on the whole against the extensive metabolism of CB3717 in the mouse. Thus, over the period 0.5–6 hours the plasma levels of CB3717 and ^{14}C were superimposable. Furthermore, most of the ^{14}C administered was excreted in the urine as CB3717 or in the feces as CB3717 and CB3751. Although extensive host metabolism of CB3717 has been excluded, these studies have not addressed the possibility of CB3717 polyglutamation. It has recently been shown that polyglutamate derivatives of CB3717 are more potent inhibitors of thymidylate synthase than the parent compound (18) and hence, we are currently seeking evidence for their formation in vivo using high-specific-activity ^{3}H-CB3717.

By comparison of the CB3717 plasma levels observed in the present study (following 100 mg/kg of CB3717) with the concentrations which produce cytotoxicity in vitro (ie, 1–10 μM) (1,3–5), it is clear that cytotoxic levels are achieved in vivo. This is consistent with the observation that this dose of CB3717 given daily for 5 days results in antitumor activity in a CB3717-sensitive tumor model (8). Therefore, the similarity of cytotoxic levels in vitro and in vivo and the lack of extensive host metabolism implicates CB3717 as the active species in the whole animal. Thus, CB3717 antitumor activity probably results solely from the inhibition of thymidylate synthase, a suggestion supported by the ability of thymidine to reverse both CB3717 cytotoxicity in vitro (1,3) and antitumor activity in vivo (8). On the basis of these data, it is concluded that the clinical trial of CB3717 currently under way represents the least ambiguous evaluation of thymidylate synthase inhibition in man yet performed. As such, these studies are an important contribution to the antimetabolite approach to cancer chemotherapy.

REFERENCES

1. JONES TR, CALVERT AH, JACKMAN AL, ET AL. A potent antitumour quinazoline inhibitor of thymidylate synthetase: synthesis, biological properties and therapeutic results in mice. Eur J Cancer 17:11–19, 1981.
2. JACKMAN AL, CALVERT AH, HART LI, ET AL. Inhibition of thymidylate synthetase by the new quinazoline antifolate CB3717; enzyme purification and kinetics. *In* Purine Metabolism in Man—IV Part B: Biochemical, Immunological and Cancer Research (De Bruyn C, Simmonds H, and Miller M, eds). New York, Plenum Press, 1984, pp 375–378.
3. JACKSON RC, JACKMAN AL, and CALVERT AH. Biochemical effects of a quinazoline inhibitor of thymidylate synthetase, *N*-(4-*N*-((2-amino-4-hydroxy-6-quinzolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid (CB3717) in human lymphoblastoid cells. Biochem Pharmacol 32:3783–3790, 1983.
4. DIDDENS H, NIETHAMMER D, and JACKSON RC. Patterns of cross-resistance to the antifolate drugs trimetrexate, metoprine, homofolate and CB3717 in human lymphoma and osteosarcoma cells resistant to methotrexate. Cancer Res 43:5286–5292, 1983.
5. NAIR MG, SALTER DC, KISLUIK RL, ET AL. Folate analogues 21. Synthesis and antifolate and antitumour activities of N^{10}-(cyanomethyl)-5,8-dideaza-folic acid. J Med Chem 26:605–607, 1983.
6. JACKMAN AL, CALVERT AH, TAYLOR GA, ET AL. Biological properties of the new quinazoline inhibitor of thymidylate synthetase, CB3717. *In* The Control of Tumour Growth and Its Biological Bases (Davis W, Maltoni C, and Tanneberger ST, eds). Berlin, Akademie Verlag, 1983, pp 404–410.
7. ALISON DL, ROBINSON BA, HARLAND SJ, ET AL. Phase I clinical trial of CB3717 (*N*-(4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)-methyl)prop-2-ynylaminobenzoyl)-L-glutamic acid). Br J Cancer 50:242–243, 1984.
8. JACKMAN AL, TAYLOR GA, CALVERT AH, ET AL. Modulation of antimetabolite effects. Effects of thymidine on the efficacy of the quinazoline-based thymidylate synthetase inhibitor, CB3717. Biochem Pharmacol 33:3269–3275, 1984.
9. SMITH RG, PEGUES JC, FARQUHAR D, ET AL. Mass spectrometry

of permethylated folic acid analogues. Biomed Mass Spectrom 8:144–148, 1981.
10. DURST T. Dimethylsulphoxide (DMSO) in organic synthesis. *In* Advances In Organic Chemistry—Methods and Results, Vol 6 (Taylor EC, and Wynberg H, eds). New York, Interscience, 1969, pp 285–338.
11. ZAHARKO DS, BRUCKNER H, and OLIVERIO VT. Antibiotics alter methotrexate metabolism and excretion. Science 166:887–888, 1969.
12. NEWELL DR, SIDDIK ZH, CALVERT AH, ET AL. Pharmacokinetics and toxicity studies with CB3717. Proc Am Assoc Cancer Res 23:181, 1982.
13. ALISON DL, NEWELL DR, SESSA C, ET AL. The clinical pharmacokinetics of the novel antifolate N^{10}-propargyl-5,8-dideazafolic acid (CB3717). Cancer Chemother Pharmacol 14:265–271, 1985.
14. JACOBS SA, STOLLER RG, CHABNER BA, ET AL. 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high dose methotrexate. J Clin Invest 57:534–538, 1976.
15. VALERINO DM, JOHNS DG, ZAHARKO DA, ET AL. Studies on the metabolism of methotrexate by intestinal flora. 1. Identification and study of biological properties of the metabolite 4-amino-4-deoxy-N^{10}-methylpteroic acid. Biochem Pharmacol 21:821–831, 1972.
16. DONEHOWER RC, HANDE KR, DRAKE JC, ET AL. Presence of 2,4-diamino-N^{10}-methylpteroic acid after high-dose methotrexate. Clin Pharmacol Ther 26:63–72, 1979.
17. BREITHAUPT H, and KÜENZLEN E. Pharmacokinetics of methotrexate and 7-hydroxymethotrexate following infusions of high-dose methotrexate. Cancer Treat Rep 66:1733–1741, 1982.
18. CHENG Y-C, DUTSCHMAN GE, STARNES MC, ET AL. Activity of the new antifolate N^{10}-propargyl-5,8-dideazafolic acid and its polyglutamates against human dihydrofolate reductase, human thymidylate synthetase, and KB cells containing different levels of dihydrofolate reductase. Cancer Res 45:598–600, 1985.

Caloric Intake Assessment in Advanced Cancer Patients: Comparison of Three Methods[1,2]

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Malnutrition is a frequent complication of advanced cancer (1,2). Although anorexia is considered one of its major causes (3,4), very few studies have focused on a better characterization of or on the development of potential treatments for this symptom. One of the reasons for this is the absence of simple and reliable methods for the assessment of food intake. Commonly used methods for caloric intake assessment often depend on cooperation from the patients (recall or prospective records). Cooperation may be difficult for patients who have asthenia or depression or are receiving narcotics (all three are frequent events in advanced cancer patients). The purpose of the present study was to compare two commonly used methods for the assessment of food intake with a new method: the 24-hour recall method and the prospective record kept by the patient were compared with a new method, consisting of a percentual estimation of food intake by a nontrained person.

PATIENTS AND METHODS

Forty-six patients with advanced cancer who were admitted to the Cross Cancer Institute were entered in the study. All of the patients were able to read and sign a consent form without assistance, and all of them were receiving the regular hospital diet. Patient characteristics are shown in table 1. During Day 1 of the study, patients who agreed to participate were trained to keep a 24-hour record of food intake. Training consisted of a single 20-minute session with a senior dietitian that included practical examples of different meals and a checklist for the patient to review. The investigators were available from 7:00 AM to 11:00 PM during the day of the study to help patients with any problem filling out the forms. Meals were served by the investigators. Also on Day 1, three nurses and two volunteers, all of them without any previous experience in nutritional assessment, were trained to perform the percentual estimation of food intake. All nurses and volunteers had the same teaching session that lasted 20 minutes and included some practical examples of calculating how much had been consumed from a certain plate or cup, by inspecting the plate or cup before and after a meal (eg, cup of soup, 50%; chicken breast, 30%; etc).

During Day 2, all of the patients' food was carefully weighed before and after every meal and snack. The food's weight provided the actual caloric and protein intake of the patients (A). Each food was weighed separately. The menu that was served had a previous calorimetric assessment by the Dietary Department (according to Standard Food Composition tables). At

TABLE 1.—Patient characteristics*

Characteristic	
Mean age (yrs) ± SD	
Male	57 ± 21
Female	60 ± 25
Sex	
Male	24
Female	22
Primary tumor	
Lung	9
Breast	5
Lymphoma	6
Leukemia	7
Myeloma	5
Gastrointestinal tract	3
Cervix	2
Prostate	5
Kidney/bladder	2
Osteosarcoma	1
Melanoma	1
Total	46
Mean daily caloric intake ± SD	
Male	1406 ± 687
Female	1172 ± 538
Mean daily protein intake (g) ± SD	
Male	66 ± 36
Female	52 ± 30

*Unless otherwise specified, values = No. of patients.

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[2] Presented at the 21st Annual Meeting of the American Society of Clinical Oncology, Houston, May 21-23, 1985.
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TABLE 2.—Correlation between A and other methods*

No.	Method	No. of patients	Daily intake†	Correlation with A	*P* value of correlation
1	%E calorie	46	1254 ± 605 calories	0.95	< 0.001
2	%E protein	46	57 ± 31 g	0.90	< 0.001
3	P calorie	44	1268 ± 594 calories	0.84	< 0.001
4	P protein	44	53 ± 30 g	0.75	< 0.001
5	R calorie	34	1119 ± 532 calories	0.65	< 0.001
6	R protein	34	49 ± 27 g	0.63	< 0.001
7	A calorie	46	1299 ± 627 calories	—	—
8	A protein	46	59 ± 34 g	—	—

*1 vs 5, $P < 0.001$; 2 vs 6, $P < 0.001$; 1 vs 3, $P < 0.001$; and 2 vs 4, $P < 0.01$.
†Values = mean ± SD.

the same time, the patient kept a 24-hour record of food intake (P) and a nurse or a volunteer calculated the percentage of food intake by inspecting the patient's dish before and after each meal (%E). Each food remaining on the plate was calculated separately.

During Day 3, a senior dietitian performed a 24-hour recall of food intake in all patients (R). All of the information was obtained and translated into calories and proteins blindly. Statistical analysis was performed using correlation tests. Difference between correlated correlations was determined as suggested by Guilford and Fruchter (5), Student's t test for the comparison of means, and the chi-square test for the comparison of proportions.

RESULTS

At the end of the study, two patients were not evaluable because their records were lost, and one patient was not evaluable for 24-hour recall (the dietitian did not perform the recall).

Eleven of 45 (24%) patients evaluated for 24-hour recall could not remember sufficient information regarding the three daily meals.

TABLE 3.—Correlation between A and %E for each of 3 nurses and 2 volunteers

Subject	Correlation with A*	*P* value
Nurse 1 ($n = 8$)		
Calories	0.95	0.001
Protein	0.96	0.001
Nurse 2 ($n = 10$)		
Calories	0.91	< 0.001
Protein	0.90	< 0.001
Nurse 3 ($n = 8$)		
Calories	0.98	< 0.001
Protein	0.98	< 0.001
Volunteer 1 ($n = 15$)		
Calories	0.96	< 0.001
Protein	0.88	< 0.001
Volunteer 2 ($n = 5$)		
Calories	0.95	< 0.01
Protein	0.80	0.05

*Correlations were not significantly different between subjects.

The correlation between A and the three different methods for food intake assessment are shown in table 2. Table 3 summarizes the correlation between A and %E when analyzed for each of the participants. The correlation between %E and A was not different when we compared solid and liquid food or when we compared different solid meals with each other.

DISCUSSION

Our results show that the 24-hour recall method is not reliable in advanced cancer patients. Twenty-four percent of the population could not recall their food intake, although they had had to record it the previous day. The rest of the patients were evaluable, but the correlation with actual food intake was poor (table 2).

The patient's record method showed better correlation than the 24-hour recall method, but it was still significantly worse than the percentual evaluation method. This percentual evaluation method is much more economically feasible and less time consuming for a dietitian than the 24-hour recall and the prospective record methods.

In outpatients, our experience suggests that the method can be successfully performed by the patient's relatives (5). Because of the fact that the method does not require the patient's cooperation and because of its simplicity, it can be repeated frequently for the assessment of changes in food intake during clinical research trials. When the patients are served different meals, the results can be expressed as a percentage of the served ration. When the patients are served the same meals in each opportunity, the results can be accurately translated into proteins and calories.

Although further research on caloric intake assessment continues to be badly needed, our results suggest that the method of percentual evaluation can provide reliable and economic information on patients' food intake.

REFERENCES

1. THEOLOGIDES A. Cancer cachexia. Cancer (suppl)63:2004-2012. 1979.
2. DEWYS W, BEGG C, CAVIN P, ET AL. Prognostic effect of weight

loss to chemotherapy in cancer patients. Am J Med 69:491–492, 1980.
3. BRUERA E, CARRARO S, ROCA E, ET AL. Association between malnutrition and caloric intake, emesis, psychological depression, glucose taste, and tumor mass. Cancer Treat Rep 68:873–876, 1984.
4. DEWYS W. Anorexia as a general side effect of cancer. Cancer (suppl)63:2013–2019, 1979.
5. GUILFORD JP, and FRUCHTER B. Fundamental Statistics in Psychology and Education. New York, McGraw Hill, 1973, pp 166–167.
6. BRUERA E, ROCA E, CEDERO L, ET AL. Action of oral methylprednisolone in terminal cancer patients: a prospective randomized double-blind study. Cancer Treat Rep 69:751–754, 1985.

Investigation of the Additive Potential of Teniposide and Vincristine in Non-Hodgkin's Lymphoma[1,2]

Australian and New Zealand Lymphoma Group[3,*]

Two hundred forty-seven patients with non-Hodgkin's lymphoma were randomized to receive doxorubicin, cyclophosphamide, and prednisolone in combination with vincristine (ACVP) or teniposide (ACTP) or both vincristine and teniposide (ACTVP). ACVP produced 44% complete remissions (CRs) and 32% partial remissions (PRs), whereas ACTP produced 46% CRs and 33% PRs and ACTVP produced 47% CRs and 36% PRs. Survival and relapse-free survival were also comparable for the three arms. The majority (59%) of patients had diffuse large cell lymphoma. Within this group, CR was achieved in 52% and no significant difference was observed in the response rates, survival, or relapse-free survival for the three treatment arms. Toxic effects occurred with equivalent frequency in the three regimens, except for neurotoxicity and myelosuppression. Moderate to severe neurotoxicity occurred in 20% of ACTVP-treated patients and in 8% of ACVP-treated patients but was not seen in any of the ACTP-treated patients ($P = 0.0004$). Severe myelosuppression occurred in 27% of ACTVP-treated patients and in 22% of ACTP-treated patients but only in 8% of ACVP-treated patients ($P = 0.02$), indicating an increased risk of myelosuppression for the combinations including teniposide. These results confirm our previous findings that teniposide can replace vincristine in the treatment of non-Hodgkin's lymphoma, with freedom from neurotoxicity and comparable survival and response rates. However, the combined use of teniposide and vincristine in the schedule as described has not increased response rates or survival but has instead added to neurotoxicity. [Cancer Treat Rep 70:985-990, 1986]

A previous study by the Australian and New Zealand Lymphoma Group (1) demonstrated that teniposide can replace vincristine in combination with cyclophosphamide and prednisolone in the treatment of non-Hodgkin's lymphoma (NHL) with freedom from neurotoxicity and comparable response and survival rates. There was also a suggestion from crossover studies that some cases of NHL did not display cross resistance to both agents, since three of 39 patients who failed to respond adequately to one regimen responded to the alternative regimen (1). The potential thus existed for both agents together to increase the therapeutic efficacy of drug combinations in the treatment of NHL without adding to myelotoxicity. We report the findings of a randomized prospective study comparing the response to ACTVP (doxorubicin, cyclophosphamide, teniposide, vincristine, and prednisolone) with that of ACTP (doxorubicin, cyclophosphamide, teniposide, and prednisolone) and ACVP (doxorubicin, cyclophosphamide, vincristine, and prednisolone) in patients with NHL.

PATIENTS AND METHODS

Eligibility Criteria

Patients between 14 and 70 years of age with a diagnosis of diffuse poorly differentiated lymphoma, nodular

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[2] Supported by the Cancer Institute, Melbourne; the Australian Cancer Society; Bristol Myers Co, Pty, Ltd; and the New Zealand Cancer Society.

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TABLE 1.—Patient characteristics*

	ACTVP	ACTP	ACVP
Total	119	64	64
Median age (yrs)	56	57	58
Sex (M:F)	70:49	38:26	32:32
Therapy			
No previous treatment	81 (68%)	48 (75%)	51 (80%)
Previous radiotherapy	18 (15%)	10 (16%)	4 (6%)
Previous chemotherapy	14 (12%)	5 (8%)	5 (8%)
Both	6 (5%)	1 (2%)	4 (6%)
Stage			
I	11 (9%)	4 (6%)	9 (14%)
II	27 (23%)	9 (14%)	9 (14%)
III	15 (13%)	12 (19%)	10 (16%)
IV	66 (55%)	39 (61%)	36 (56%)
B symptoms	39 (33%)	30 (47%)	26 (41%)

*Unless otherwise specified, values = No. of patients.

and diffuse mixed cell lymphoma, and nodular and diffuse large cell lymphoma were eligible. Patients who had received prior radiotherapy and/or chemotherapy were included, provided this had not been administered in the preceding 4 weeks and chemotherapy had not included doxorubicin. A measurable lesion was not considered essential for entry. Informed consent was obtained from all patients.

Between February 1979 and November 1981, 288 patients were randomized on the basis of an appropriate diagnosis by the participating institution. All biopsy specimens were reviewed by a panel of three histopathologists using a modified Rappaport system (2,3), and 38 were excluded on the basis of this review. Three other patients were excluded: one because of age, one because of cardiac disease, and one because of inadequate investigation prior to randomization. All subsequent results refer to the remaining 247 patients.

Patient Characteristics

Patient characteristics are shown in table 1. The median age was 57 years, and the male:female ratio was 140:107. Most of the patients (73%) had received no previous treatment, 13% had received radiotherapy only, 9% had received chemotherapy only, and an additional 4% had received both chemotherapy and radiotherapy prior to randomization. The majority of patients had advanced disease: 10% were Stage I, 18% were Stage II, 15% were Stage III, and 57% were Stage IV, with a similar distribution between the randomization arms.

Staging

Investigations to determine the stage of disease included measurements of accessible lesions, chest x-ray, cbc, blood biochemistry, percutaneous bone marrow trephine biopsy (99% compliance), bilateral lower limb lymphangiography (78%), abdominal computerized tomographic scan (73%), radionuclide liver spleen scan (51%), and inferior venacavography (39%). The extent of disease was classified by Ann Arbor Conference criteria (4).

Therapy

Of the 247 eligible patients, 119 were randomized to receive ACTVP, 64 to receive ACTP, and 64 to receive ACVP according to dose levels shown in table 2. Treatment was started at Level B in 236 patients and at Level C in 11 patients. Dosage for subsequent courses was adjusted according to the level of both the neutrophil and platelet counts at the time treatment was due, as specified in table 2. One hundred eighty-eight patients (76%) completed a minimum of six courses of treatment; of these, 117 patients (62%) received > 75% of the ideal dosage, corresponding to the initial dose at Level B followed by five courses at Level A.

Courses were repeated at 3-week intervals for a minimum of three courses at the end of which patients were reassessed clinically and by repeating the investigations that initially showed abnormal results. All patients achieving complete remission (CR; no evidence of disease activity) received a further three cycles every 3 weeks as consolidation. The patients achieving partial remission (PR; decrease in size of > 50% of measurable lesions, no recurrences, and no new lesions) continued on 3-week cycles until CR was achieved, or until the maximum recommended dose of doxorubicin (450 mg/m^2) had been reached.

Statistical Considerations

Patients were randomized to the three treatment arms ACTVP, ACTP, and ACVP in the ratio 2:1:1, respectively, anticipating from the previous study that the two arms ACTP and ACVP would produce equivalent results with respect to response and survival, and hence could be merged at the time of analysis if required. Prior to randomization, patients were stratified by the participating centers.

It was originally planned to randomize 200 eligible patients. Patient entry continued for a short time beyond

TABLE 2.—Dose schedule

Neutrophil count ($\times 10^9$ cells/L)	Platelet count ($\times 10^9$ cells/L)	Level	Dose—				
			Doxorubicin	Vincristine	Teniposide	Cyclophosphamide	Prednisolone
> 2	> 100	A	50 mg/m^2	1.4 mg/m^2	100 mg/m^2	750 mg/m^2	100 mg
1-2	50-100	B	35 mg/m^2	1.4 mg/m^2	75 mg/m^2	400 mg/m^2	100 mg
< 1	< 50	C	25 mg/m^2	1.4 mg/m^2	50 mg/m^2	200 mg/m^2	100 mg

that target while plans for a subsequent study were in progress. Results were reviewed at 6-month intervals corresponding to participants' meetings. This review had no impact on the overall significance of the results, since it was predetermined to stop the trial only if the significance level of differences in CR between different treatment arms fell to < 0.01 at an interim assessment.

Twelve patients (five receiving ACTVP; four receiving ACTP; and three receiving ACVP) have been lost to follow-up after a median period of 10 months (range, 1–22). Eight of these 12 patients were disease-free at the date of last contact, two had disease present, and two were awaiting assessment. For determination of survival these patients have been censored at the date of last contact. Similarly, seven patients (one receiving ACTVP; four receiving ACTP; and two receiving ACVP) who died of other causes without disease present have been censored at the date of death. Inclusion of these patients as deaths due to the disease did not alter the conclusions drawn. Thirteen patients in whom all evaluable disease was absent because of surgical resection at the time of diagnosis were excluded from evaluation for induction of CR, but were included in the study of overall survival.

Actuarial survival curves were calculated using 1-month intervals. Survival and relapse-free survival were measured from the randomization date. The log-rank test was used to measure the significance of differences in the survival and relapse experience between groups. Comparisons of the randomization arms were made separately within each of the five histological subgroups and combined to give an overall comparison adjusting for histology (5,6). P values were determined by two-tailed significance tests.

Relative death rates and relative relapse rates have been chosen as summary statistics. In comparing the survival or relapse experiences of two or more groups, the relative death or relapse rate for any one group can be interpreted as the ratio O/E, where O is the observed number of deaths or relapses in that group and E is the extent of exposure to the risk of death or relapse for that group calculated under the null hypothesis that there is no difference between the groups.

RESULTS

Induction of CR

CR was achieved in 107 (46% ± 3% SE) of the 234 patients with evaluable disease. No significant difference was observed in the response to the different regimens: CR was achieved in 47% (±5% SE) of ACTVP-treated patients, in 46% (±6% SE) of ACTP-treated patients, and in 44% (±6% SE) of ACVP-treated patients. The increase in response rate achieved using ACTVP rather than ACTP or ACVP was 2% (95% confidence interval, −11% to +15%). The response rates within the different histological types are shown in table 3.

Relapse-Free Survival

The relapse-free survival of the 107 patients who achieved CR is shown in figure 1. Overall, the estimated relapse-free survival rate is 46% at 5 years (±5% SE). There was no statistically significant difference in the relapse-free survival experience between the three treatment arms, with estimated 5-year relapse-free survival rates of 45% (±7% SE), 56% (±12% SE), and 38% (±11% SE) for ACTVP-, ACTP-, and ACVP-treated patients, respectively. The relative relapse rates were 1.11, 0.71, and 1.10 for ACTVP-, ACTP-, and ACVP-treated patients, respectively, adjusting for differences in histology ($P = 0.4$).

Survival

Actuarial survival of all eligible patients is shown in figure 2, with an estimated 44% (±4% SE) of patients surviving 5 years from the date of randomization. The outcome was very similar for each treatment group, with estimated 5-year survival rates of 45% (±5% SE), 46% (±8% SE), and 39% (±8% SE) for ACTVP-, ACTP-, and ACVP-treated patients, respectively. The relative death rates were 0.98, 0.97, and 1.08 for ACTVP-, ACTP-, and ACVP-treated patients, respectively, adjusting for differences in histology ($P = 0.9$).

TABLE 3.—Incidence of CR*

Tumor type	Proportion of patients achieving CR†				
	ACTVP	ACTP	ACVP	Total	% CR
Diffuse poorly differentiated lymphoma	6/19	2/13	6/11	14/43	33
Nodular mixed cell lymphoma	3/8	1/6	2/6	6/20	30
Diffuse mixed cell lymphoma	7/13	5/8	4/11	16/32	50
Nodular large cell lymphoma	1/3	0/0	0/1	1/4	25
Diffuse large cell lymphoma	35/68	20/34	15/33	70/135	52
Total	52/111	28/61	27/62	107/234	
% CR	47	46	44	46	

*Values = No. of patients with CR/total No.

†13 patients with no evidence of disease at randomization have been excluded from this table.

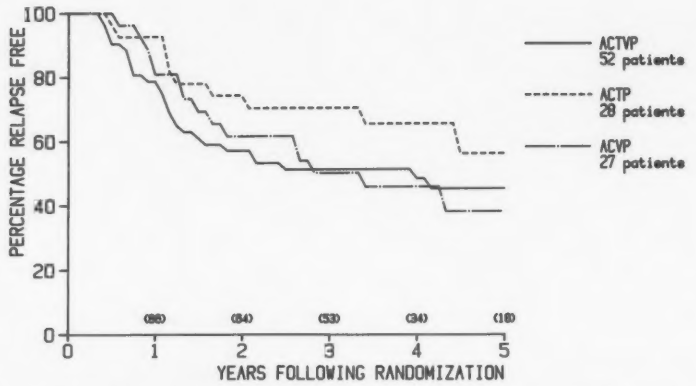

FIGURE 1.—Relapse-free survival by randomization arm. Values in parentheses = No. of patients at risk at beginning of the period.

Diffuse Large Cell (DLC) Lymphoma Patients

One hundred forty-six patients (59%) in this study had a diagnosis of DLC. Seventy-three percent had received no prior treatment, 14% had received radiotherapy alone, 10% had received chemotherapy alone, and an additional 3% had received both. The majority of these patients had advanced disease: 17% had Stage I, 21% had Stage II, 14% had Stage III, and 53% had Stage IV. Thirty-seven percent also had constitutional symptoms. Eleven patients had no evaluable disease at the time of randomization. For the remaining 135 patients, 52% (±4% SE) achieved CR (table 3). No significant difference was observed in the response rates for the three randomization arms, CR being achieved in 51% (±6% SE) of ACTVP-treated patients, in 59% (±8% SE) of ACTP-treated patients, and in 49% (±9% SE) of ACVP-treated patients.

The relapse-free survival experience for the 70 patients with DLC lymphoma who achieved CR is shown in figure 3, with an estimated 55% (±6% SE) of these patients remaining relapse-free 5 years after randomization. The relative relapse rates were 1.1, 0.7, and 1.2 for ACTVP-, ACTP-, and ACVP-treated patients, respectively ($P = 0.5$).

The actuarial survival of the 146 patients with DLC lymphoma is shown in figure 4; an estimated 42% (±4% SE) of the patients were alive 5 years after randomization. The relative death rates were 1.1, 0.8, and 1.2 for ACTVP-, ACTP-, and ACVP-treated patients, respectively ($P = 0.4$).

Patients With No Prior Therapy

The majority of patients (76%) had had no prior therapy. Within this group of patients and the subgroup of patients with DLC lymphoma, there was no difference in the CR rates, relapse-free survival, and the overall survival for the three treatment arms.

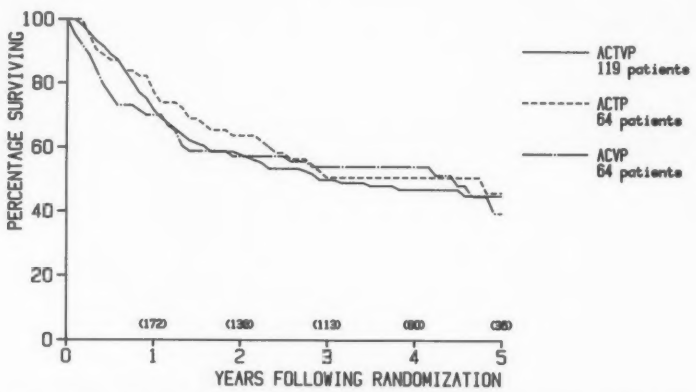

FIGURE 2.—Overall survival by randomization arm. Values in parentheses = No. of patients at risk at beginning of the period.

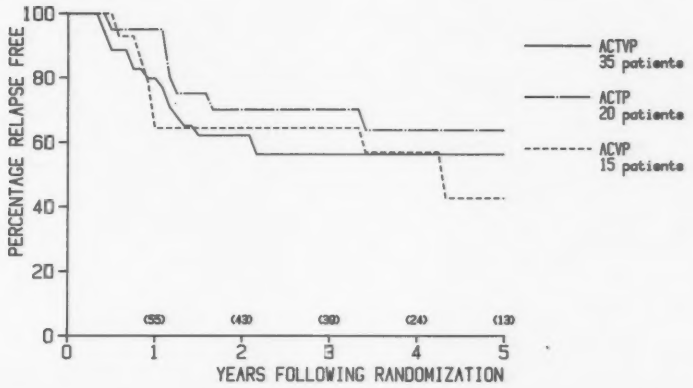

FIGURE 3.—Relapse-free survival of patients with DLC lymphoma. Values in parentheses = No. of patients at risk at beginning of the period.

Toxicity

Toxicity was assessed by the attending physician before the beginning of each cycle of treatment and graded using the criteria shown in tables 4 and 5. The degrees of hematopoietic and neurological toxicity experienced by patients in the three treatment arms are shown in table 4.

Marked myelosuppression (grade 3) was seen in 27% of patients receiving ACTVP and in 22% of patients receiving ACTP in contrast to 8% of patients receiving ACVP ($P = 0.02$). This was also reflected in the average dose level of drugs given ($P = 0.13$).

Grade 1 neurologic toxicity was recorded in 42% of ACTVP-, in 52% of ACVP-, and in 16% of ACTP-treated patient groups. More marked neurologic toxicity (grades 2 and 3) was seen in 20% of ACTVP-, in 8% of ACVP-, and in 0% of ACTP-treated patients ($P = 0.0004$). All other toxic effects were equal.

DISCUSSION

In an earlier randomized study of 164 patients with a wide variety of NHL the Australian and New Zealand Lymphoma Group showed that the response and survival rates with teniposide and vincristine were comparable when each was used in combination with cyclophosphamide and prednisolone (1). That study also demonstrated that teniposide was free from neurotoxicity whereas vincristine caused moderate to severe symptoms in almost half the patients treated. The avoidance of these unpleasant and sometimes disabling symptoms without loss of efficacy seemed a significant advance.

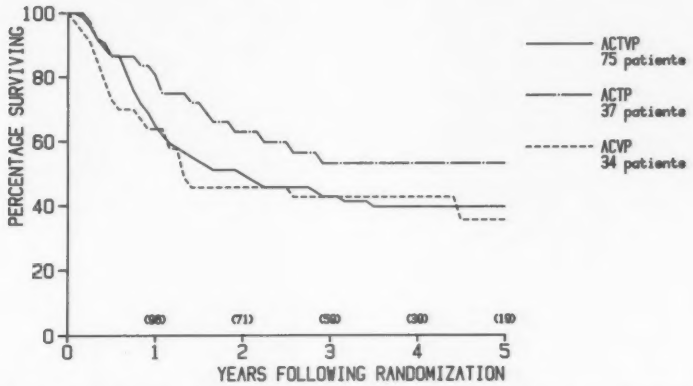

FIGURE 4.—Overall survival of patients with DLC lymphoma. Values in parentheses = No. of patients at risk at beginning of the period.

TABLE 4.—Hematopoietic and neurologic toxicity

Treatment arm (No. of patients)	Median No. of courses	% of patients receiving an average of ≥ 75% ideal dose	% of patients in each toxicity grade							
			Hematopoietic*				Neurologic†			
			0	1	2	3	0	1	2	3
ACTVP (119)	6	47	12	22	40	27	39	42	19	1
ACTP (64)	6	53	19	23	36	22	84	16	0	0
ACVP (64)	6	70	25	27	40	8	40	52	6	2

*For hematopoietic toxicity grades *see* table 5.

†Neurotoxicity grades: 0 = normal; 1 = reduced tendon reflexes and/or paresthesia; 2 = absent tendon reflexes and 25% motor loss; and 3 = incapacitating weakness.

TABLE 5.—Hematopoietic toxicity grades

	Grade—			
	0	1	2	3
Hemoglobin (g/dl)	> 10	9.0–9.9	7.0–8.9	< 7.0
Granulocyte count ($\times 10^9$ cells/L)	> 2	1.0–1.99	0.5–0.99	< 0.5
Platelet count ($\times 10^9$ cells/L)	> 100	50–99	25–49	< 25

Towards the end of the first study it appeared from the literature that patients with lymphoma of unfavorable histology fared better when treated with anthracycline-containing drug combinations (7–10). In planning the present study we therefore added doxorubicin to CTP and CVP and incorporated a third arm which included both vincristine and teniposide (ACTVP) to test whether there might be an added antitumor effect. It had been hoped that the different pharmacodynamics and mechanisms of action of teniposide relative to vincristine would augment the overall effect (11,12). The results in this study showed that the addition of vincristine to teniposide given in the schedule described has failed to enhance either response rates or survival. The CR rate of 52% in DLC lymphoma is nevertheless comparable to the results of several published studies using various anthracycline-containing combinations (7–10). In combination with doxorubicin, cyclophosphamide, and prednisolone, teniposide appears to result in increased myelosuppression. When given with vincristine, however, it has increased neurotoxicity; grade 2 and 3 toxicity were seen in 20% of patients receiving both vincristine and teniposide (ACTVP) in contrast to 8% of those receiving vincristine (ACVP) and 0% of those receiving teniposide (ACTP).

The study again confirms our previous findings of the absence of any severe neurotoxicity with teniposide, and that teniposide produces comparable response and survival rates to vincristine in NHL when used in combination with cyclophosphamide and prednisolone, this time with the addition of doxorubicin. We have thus demonstrated in two consecutive studies that teniposide has a definite place in the management of NHL because of its lack of severe neurotoxicity and its ability to replace vincristine in combination chemotherapy without loss of efficacy.

REFERENCES

1. The Australian and New Zealand Lymphoma Co-operative Chemotherapy Study Group. Comparison of the use of teniposide and vincristine in combination chemotherapy for non-Hodgkin's lymphoma. Cancer Treat Rep 66:49–55, 1982.
2. RAPPAPORT H. Atlas of Tumor Pathology, section III, fascicle 8. Washington, DC, US Armed Forces Institute of Pathology, 1966.
3. NATHWANI BN, KIM H, RAPPAPORT H, ET AL. Non-Hodgkin's lymphomas: a clinicopathologic study comparing two classifications. Cancer 41:303–325, 1978.
4. CARBONE PP, KAPLAN HS, MUSSHOFF K, ET AL. Report of the committee on Hodgkin's disease staging. Cancer Res 31:1860–1861, 1971.
5. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. 1. Introduction and design. Br J Cancer 34:585–612, 1976.
6. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient II. Analysis and examples. Br J Cancer 35:1–39, 1977.
7. SCHEIN PS, DEVITA VT, JR, HUBBARD S, ET AL. Bleomycin, adriamycin, cyclophosphamide, vincristine and prednisolone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85:417–422, 1976.
8. SKARIN AT, ROSENTHAL DS, MOLONEY WC, ET AL. Combination chemotherapy of advanced non-Hodgkin's lymphoma with bleomycin, adriamycin, cyclophosphamide, vincristine and prednisolone (BACOP). Blood 49:759–770, 1977.
9. LISTER TA, CULLEN MH, BREARLEY RB, ET AL. Combination chemotherapy for advanced non-Hodgkin's lymphoma of unfavourable histology. Cancer Chemother Pharmacol 1:107–112, 1978.
10. BENNETT JM, LENHARD RE, JR, EZDINLI E, ET AL. Chemotherapy of non-Hodgkin's lymphomas: Eastern Cooperative Oncology Group experience. Cancer Treat Rep 61:1079–1083, 1977.
11. OWELLEN RJ, HARTKE CA, DICKERSON RM, ET AL. Inhibition of tubulin-microtubule polymerisation by drugs of the vinca alkaloid class. Cancer Res 36:1499–1502, 1976.
12. DREWINKO B, and BARLOGIE B. Survival and cycle-progression delay of human lymphoma cells in vitro exposed to VP-16-213. Cancer Treat Rep 60:1295–1306, 1976.

Hexamethylene Bisacetamide: A Polar-Planar Compound Entering Clinical Trials as a Differentiating Agent[1]

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There is evidence that a number of peptide growth factors and low molecular weight chemicals can induce differentiation of several tumor cell lines (1-3). These include peptide growth factors, vitamins, hormones, chemotherapeutic agents, and polar-planar solvent. Once differentiated, tumor cells cease to proliferate and lose their clonogenicity in soft agar and their ability to grow when transplanted into animals. Although the precise mechanism of action is yet unknown, these inducing agents appear to control the expression of specific genes, including both protogenes and oncogenes (1). Conceptually, such agents could prevent the progression of a premalignant cell to a malignant state, or in certain cases, they could arrest or reverse the process of malignant transformation (1).

Hexamethylene bisacetamide (HMBA) is a polar-planar compound structurally related to dimethyl sulfoxide (DMSO) and *N*-methylformamide (NMF) (fig 1). The ability of DMSO to induce erythroid differentiation in virus-infected murine erythroleukemia cells (MELC) was first observed by Friend et al in 1971 (4). This observation led Marks and associates to examine DMSO and other polar-planar compounds in an attempt to understand the molecular biology of the induction process and to identify more potent agents. After extensive structure-activity studies (5-12), HMBA was identified as the most potent inducer for the erythroid differentiation of MELC in this chemical class (6). Subsequently, the compound was found to induce differentiation in a number of cell types including human HL-60 promyelocytic leukemia cells (13). Preliminary data also suggest that HMBA inhibits tumor induction (14). However, in contrast to other agents which show ability to induce differentiation (cytarabine, dactinomycin, NMF, etc), HMBA does not exhibit any antitumor activity in conventional in vivo murine tumor models used for routine screening (15). In addition, the compound is not cytotoxic at concentrations that induce differentiation. The fact that HMBA is an effective inducer of differentiation at noncytotoxic concentrations makes it a good candidate for evaluating the selective efficacy of a differentiating agent in human cancer. For this reason, HMBA was chosen by the National Cancer Institute (NCI) for further development and is now being introduced into clinical studies.

PRECLINICAL STUDIES

In Vitro Studies

At a concentration of 5 mM for 5 days, HMBA induced > 99% of the MELC to differentiate and produce hemoglobin, a product formed only in fully differentiated cells (6). Higher concentrations of HMBA were cytotoxic. HMBA-induced erythroid differentiation was accompanied by morphological and biochemical changes that resemble normal murine erythroid differentiation (6,9). HMBA was more potent and more efficacious in inducing differentiation of MELC than other polar-planar compounds (table 1). Similarly, HMBA was the most potent inducer among seven hexamethylenebisamide analogs evaluated (16). When MELC were exposed to HMBA at various concentrations and periods, the proportion of cells to differentiate was dependent on both concentration and duration of exposure to the agent. Concentrations of the agent required for 90% of the MELC to be stained positive with benzidine (a biochemical test for hemoglobin) were 3 and 2 mM for 3- and 6-day cultures, respectively (table 2) (17). More than 60% of the cells were induced to differentiate at a concentration of 1 mM when the exposure time was extended to 6-9 days. Under suboptimal conditions, the induced differentiation was not fully stabilized and a single cell could give rise to a colony containing both differentiated and undifferentiated cells (7).

The evidence that HMBA is an effective inducer of differentiation is not limited to studies in MELC. HMBA

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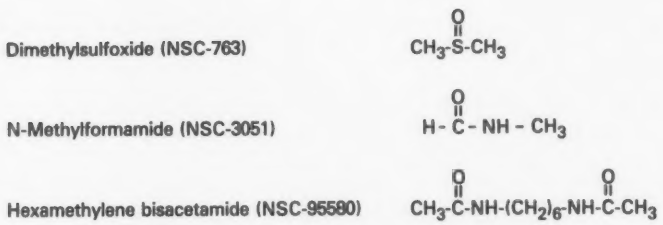

FIGURE 1.—Comparative structures of polar-planar compounds.

was the most efficacious among a series of agents examined for the ability to induce differentiation of human HL-60 promyelocytic leukemia cells (13). In this study, HMBA induced 95% of the promyelocytic leukemia cells to form mature myeloid cells after 6 days at a concentration of 2 mM. Mouse neuroblastoma cells exposed to HMBA developed extended neurites and an excitable membrane (18). Addition of HMBA to cultures of a malignant mesenchymal cell line derived from a human glioblastoma multiforme induced morphologic changes and stimulated the synthesis of procollagen (19). One of several embryonal carcinoma cell lines (PCC3/A/1) isolated from mouse teratocarcinoma exhibited morphological, biochemical, and immunological changes on exposure to HMBA; these included the induction of tropomyosin synthesis and the formation of actin filaments (20,21). The compound also markedly stimulated the formation of "domes" by the epithelial cell line MDCK (Madin-Darby canine kidney); these fluid-filled domes or hemicysts are a manifestation of specialized epithelial transport phenomenon (22). In addition, HMBA inhibited fusion of rat L8 myoblasts into myotubules and the cells assumed a fibroblast-like morphology; this alteration in phenotype was accompanied by the induction of collagen synthesis and its secretion (11,12). In all of these systems, activity was observed in the same range of concentrations (1–5 mM) effective in MELC differentiation.

TABLE 1.—Comparison of several differentiation inducers at optimal concentrations in the Friend murine erythroleukemia system*

Inducer	Optimal concentration (mM)	Cells/ml ($\times 10^6$)	% benzidine-positive
DMSO	280	1.2	67
N-Methylacetamide	30	1.1	87
N,N-Dimethylacetamide	20	1.0	73
HMBA	5	1.6	99
Butyric acid	1	1.2	42
Control	None	1.4	0.5

*Cultures of Friend MELC (strain 745A) were grown in the presence of several inducing agents. On Day 5, the % of cells which were benzidine-reactive (ie, hemoglobin-producing) was determined. Since the cells grew logarithmically for only 2–3 days and then entered stationary phase, cultures were seeded at 1×10^5 cells/ml in the presence of each inducer, and every 2nd day were diluted back to the initial cell density in the presence of the same concentration of inducer (6).

In the human tumor colony-forming assay (23), a continuous exposure to 5 mM HMBA reduced colony formation to ≤ 30% of that in controls in seven of 16 fresh surgical tumor specimens (none of two lung cancer specimens, one of one Hodgkin's disease specimens, one of four melanoma specimens, four of seven ovarian cancer specimens, and one of two other specimens). Whether the inhibitory effect on colony formation resulted from cytotoxicity or induction of differentiation cannot be ascertained without additional studies.

In Vivo Studies

In in vivo studies employing the NCI's murine and xenograft screening tumors, HMBA exhibited no antitumor activity against P388 and L1210 leukemias, B16 melanoma, C38 colon, $CD8F_1$ mammary and Lewis lung carcinomas, M5076 sarcoma, or xenografts of human tumors (MX-1 mammary, CX-1 colon, and LX-1 lung carcinomas) (15). Neither cytotoxicity nor differentiating activity of the compound against these tumor cells has been evaluated in vitro.

HMBA-induced differentiation of MELC appears to be associated with a loss of oncogenicity. While inoculation of 2×10^5 MELC to susceptible mice uniformly caused death within 4 weeks, inoculation of up to 1×10^6 cells precultured for 5 days with HMBA was not associated with a fatal outcome in recipient mice (9). Nevertheless, HMBA was not effective in prolonging the survival time of mice bearing sc implanted murine Friend erythroleukemia (9,10).

The inhibitory effect of HMBA on tumor induction was studied in an experiment in which female Sprague-Dawley rats received methylnitrosourea as a carcinogen (14). Daily ip treatments of HMBA over a dose range of 250–1500 mg/kg began 1 week later and continued for 140 days after the carcinogen. The saline-treated control group developed 39 tumors, while rats receiving HMBA at doses of either 1000 or 1500 mg/kg/treatment developed nine tumors per group (table 3). By 125 days after carcinogen, one tumor in the 1000-mg/kg treatment group and two in the 1500-mg/kg treatment group began to regress. These preliminary results indicate that HMBA may alter the process of tumorigenesis and suppress the development of a malignant phenotype.

Recently, a synergism between HMBA and the cytotoxic agent cyclophosphamide on the survival of athymic

TABLE 2.—Effect of concentration of HMBA for various durations on differentiation of Friend MELC*

HMBA (mM)	Day 3		Day 6		Day 9	
	Cells ($\times 10^6$/ml)	% benzidine-positive	Cells ($\times 10^6$/ml)	% benzidine-positive	Cells ($\times 10^6$/ml)	% benzidine-positive
0	2.5	0	2.5	0	2.6	0
1.0	2.5	25	2.2	70	2.1	63
1.5	2.5	60	1.9	80	0.9	85
2.0	2.6	76	1.2	91	0.5	94
3.0	2.6	90	0.55	98	0.4	90
4.0	2.3	90	0.44	98	0.4	90
5.0	2.2	90	0.45	98	0.4	93

*Cells (clone DS-19) were transferred every 3 days at an initial cell concentration at each passage of 10^5/ml. At each passage cells were exposed to fresh medium containing HMBA at concentrations from 1 to 5 mM. At passage, cells were counted and stained for benzidine-reactive cells (17).

nude mice implanted with ascitic HL-60 human promyelocytic leukemia has been suggested (24).

MECHANISM OF ACTION

The precise mechanism of action of HMBA as an inducer of differentiation remains unknown at the present time. Studies on the mechanism of action of HMBA have been conducted exclusively in MELC. Studies on the structure-activity relationships of various polar-planar compounds have demonstrated that the activity is derived from the Lewis base character of the CH_3-CO-NH moiety (16), and that the optimal number of carbon atoms between the functional polar groups is five or six for maximum differentiating activity (8). Furthermore, to be active as a differentiation inducer, a polar-planar compound must be relatively flexible and must penetrate the cell membrane (10).

When the cellular uptake, metabolism, and intracellular distribution of HMBA were studied using radioactive labels in various portions of the molecule, the compound was taken up by the cell; an intracellular concentration equal to the extracellular concentration was achieved by 6–8 hours of exposure (11,12). Uptake continued for up to 20–24 hours but with no further increase in intracellular concentration of undegraded compound. Commitment to differentiation, defined as the ability to continue differentiation in the absence of inducer after an initial exposure to inducer, however, was not detected until several hours after equilibration; commitment can be first detected 12–24 hours after continuous exposure to HMBA (11,12). Data suggest that this commitment of MELC differentiation occurs in a stochastic manner with a finite number entering the "committed" state during each replication cycle and continues until essentially all cells are committed. Thereafter, the differentiation inducer can be removed and the committed cells will divide only up to six times and then lose proliferative capacity (25).

Several studies have demonstrated alterations in cell cycle kinetics with chemically induced differentiation. MELC cultured with inducing agents, including HMBA, exhibited a prolongation of the G_1 phase (26,27). Transient increase in the intracellular level of cyclic adenosine 3′:5′-monophosphate was observed prior to G_1 prolongation (28). The significance of this phenomenon remains unknown. Furthermore, the prolonged G_1 phase may not be a prerequisite for erythroid differentiation

TABLE 3.—Effect of HMBA on methylnitrosourea-induced mammary tumorigenesis in rats*

Treatment	100 days after carcinogen			120 days after carcinogen			
	Tumor No.†	Incidence (%)	Total No. of tumors	Tumor No.†	Incidence (%)	Total No. of tumors	Total No. of tumor regressions
None‡	0/10	0	0	0/10	0	0	0
Saline	8/8	100	39	8/8	100	46	0
HMBA (mg/kg/day)							
250	10/10	100	34	10/10	100	48	0
500	8/10	80	22	8/10	80	30	0
1000	5/10	50	9	7/10	70	13	1
1500	4/8§	50	9	5/8§	60	10	2

*Female Sprague-Dawley rats at 50–56 days of age received methylnitrosourea (50 mg/kg) as a single iv dose. Daily ip treatments of HMBA began 1 wk later and continued for 140 days (14).
†Values = No. of rats with tumorigenesis/total No.
‡No methylnitrosourea and no HMBA.
§2 deaths (unknown causes).

with all inducers (27). Using MELC synchronized by exposure to high levels of thymidine, it has been shown that DMSO must be present during DNA synthesis (S phase) for induction of erythroid differentiation of MELC (29). Similar findings were observed in a study with HMBA (16). However, in the HL-60 cell line, commitment to differentiation had no linkage to the cell cycle (30).

Recent data suggest that HMBA and its analogs decrease the thermostability of chromosomal DNA and dissociate a specific region of cellular DNA, leading to the commitment of cell differentiation (16). Furthermore, HMBA-mediated changes in DNA and chromatin structure near the globin genes during MELC differentiation have been described (31). These changes include the development of a new DNase I-sensitive region and S1 nuclease-sensitive site 5′ to the cap site of both the $alpha_1$ and $beta^{maj}$ genes.

The temporal sequence of the various biochemical features of induced differentiation in MELC lines may provide a clue to their role in the process. When MELC are cultured in the presence of HMBA, accumulation of alpha-globin mRNA can be detected in the cytoplasm by 12–16 hours. The fact that accumulation of alpha-globin mRNA is initiated at approximately the same time that commitment occurs suggests that commitment and accumulation of newly synthesized alpha-globin may be closely linked phenomena in the course of differentiation (11,12). Cytoplasmic accumulation of beta-globin mRNA sequences is not detected until about 8 hours later. By 48 hours there is an approximately ten-fold increase in the globin mRNA content of induced MELC. Employing the nuclear chain elongation transcription assay, it has been demonstrated that the HMBA-mediated increase in accumulation of alpha- and beta-globin mRNA reflects, primarily, control of gene expression at the level of transcription (32).

PRECLINICAL TOXICOLOGY

Preclinical toxicology studies of HMBA were conducted in Sprague-Dawley rats and beagle dogs (33).

In lethality studies, rats received ip injections of HMBA in sterile water every 4 hours for 48 hours (total, 13 doses). LD50 values were 707 mg/kg (4242 mg/m^2) every 4 hours × 13 for males and 933 mg/kg (5598 mg/m^2) every 4 hours × 13 for females. Toxic effects were observed only in rats dosed ≥ 1000 mg/kg/injection. Frequent observations of dyspnea, tremors, and convulsions suggested that the CNS is the major target organ system for toxicity.

In the rat toxicity study using an iv bolus daily × 5 schedule, the target organ systems were CNS and male reproductive organs. CNS effects were observed at doses ≥ 1000 mg/kg/day (6000 mg/m^2/day) and disappeared 3 minutes after the end of dosing. Irreversible spermatogenic granulomas of the epididymides developed in all male rats dosed ≥ 500 mg/kg/day (3000 mg/m^2/day) and led to atrophy and/or degeneration of the spermatogenic epithelium. The lowest dose at which atrophy and/or degeneration occurred was 333 mg/kg/day (1998 mg/m^2/day). No evidence of reproductive organ toxicity was found in female rats.

In toxicity studies in beagle dogs in which the compound was given by continuous infusion for 46–120 hours, CNS was again the principal target system of toxicity, characterized by convulsion, agitation, nervousness, and fine body tremors. Neurotoxicity was dose-related, reversible in surviving dogs, and was not sex-related. The toxic dose low was 20–40 mg/kg/hour (400–800 mg/m^2/hour) by 120-hour continuous infusion. However, no gross or microscopic lesions were found in the CNS. No evidence of reproductive organ toxicity was observed in the dogs.

The estimated LD50s after ip injection were 4100 mg/kg for NIH Swiss mice and 3250 mg/kg for Sprague-Dawley rats using a 7-day period of observation (34).

PHARMACOKINETICS

Pharmacokinetic data were obtained during the toxicity studies in beagle dogs. The HMBA was determined by a fused silica capillary column gas chromatography using a nitrogen-phosphorus detector (15,35). Plasma steady-state levels (Cp_{ss}) of HMBA were attained within 7–12 hours with a linear relationship between the Cp_{ss} and infusion rate. Cp_{ss} of 79.2 ± 10.6, 149.8 ± 15.4, and 211.5 ± 52.8 μg/ml (0.40, 0.75, and 1.05 mM, respectively) were achieved after doses of 20. 40, and 60 mg/kg/hour, respectively. Harmonic mean plasma half-lives, estimated during the pilot and full toxicity studies, were 113 and 106 minutes. Total plasma clearance rates were independent of dose, indicative of first-order pharmacokinetics. The apparent volumes of distribution were 67.5 and 70.9 L/kg in two successive studies. These values are slightly higher than total-body water (60% of body weight), indicating some degree of tissue binding. Renal clearance was 1.59 ± 0.36 ml/minute/kg and accounted for 40.8% (range, 25.1%–51.8%) of total-body clearance. These results suggest that the kidney is the major pathway for elimination (15,33).

Recently, the pharmacokinetics, excretion, tissue distribution, and oral bioavailability of HMBA have been studied in rats, using gas chromatography (34,35). Following a single iv bolus injection (1000 mg/kg) to rats, HMBA was removed from the plasma with a half-life of 2.2 ± 0.5 hours, and 65% ± 8% of the dose was excreted unchanged in the urine during the first 24 hours after dosing. During an 8-hour iv infusion of a dose of 4785 mg/kg of HMBA, plasma concentrations of 4 mM were easily maintained with no apparent adverse effects. The drug was uniformly distributed, with highest concentrations found in thymus, kidney, liver, and lymph nodes throughout the first 24 hours after a single iv bolus dose. In vivo metabolism was very small as determined by a gas chromatographic method (35). However, the mono- and di-amine metabolites of HMBA cannot be

measured by this method. Further studies on the metabolism of the compound with an improved assay method are in progress. A peak plasma concentration of 2 mM was achieved 1 hour after oral administration (1000 mg/kg). After oral dosing, urinary excretion and plasma decay were comparable to similar data obtained after iv dosing.

PHARMACEUTICAL DATA

HMBA appears as a white crystalline powder having a molecular formula of $C_{10}H_{20}N_2O_2$ (fig 1), with a molecular weight of 200.3. No decomposition is detected after 30 days' storage at 60°C, as assayed by high-pressure liquid chromatography. The compound is water-soluble (solubility > 50 mg/ml in distilled water) and solutions of HMBA show no chemical degradation after autoclaving.

HMBA is currently supplied for clinical trials as sterile solution for iv administration. Formulations of two concentrations are provided; 3% HMBA (30 mg/ml) in 500 ml of 0.9% sodium chloride for injection and 5% HMBA (50 mg/ml) in 500 ml of sterile water for injection. Both solutions have a pH range of 5.0–7.0, with approximate osmolarities of 450 mosm/kg for the former and 250 mosm/kg for the latter. The infusion requires the use of a self-venting administration set. The use of an in-line filter does not affect the potency of the product. Infusion bottles should be stored at controlled room temperature (15°C–30°C). However, since the solutions are terminally autoclaved during manufacture, short-term exposure to elevated temperatures should not affect the products. The addition of other medications to the infusion solution is not recommended at this time, since chemical and physical interactions have not been evaluated. Changes of formulation (including the possible development of an oral formulation) are anticipated as clinical development progresses, since a high plasma concentration for a prolonged period will probably be necessary for an adequate evaluation of efficacy in man.

CLINICAL TRIALS

Two phase I trials using a schedule of 120-hour continuous infusion have been activated and are actively in progress. The starting doses were 4800 mg/m^2/day (one-half of the toxic dose low for dogs) in both studies. The third trial evaluating a 10-day continuous infusion schedule is also in progress. These studies have two objectives: the determination of the dose-limiting toxicity and the maximally tolerated dose of HMBA together with the determination of pharmacokinetics (in particular, maximum achievable Cp_{ss} and metabolism) in humans.

DISCUSSION

Laboratory studies using in vitro systems and animal models suggested that many chemically unrelated compounds are capable of inducing tumor cell maturation, differentiation, and changes in markers reflecting a mature phenotype (1-3). These observations led to an enthusiasm by investigators for the possible clinical application of differentiating agents in the management of malignant disorders. In fact, clinical experience has already been obtained with several "differentiating agents," mainly low-dose cytotoxic antineoplastic agents. However, not only have the results been variable and controversial but also there are difficulties in evaluating the role of differentiation in the results (vide infra).

There are major problems in both preclinical and clinical development and evaluation of differentiating agents. Absence of an adequate screening system for differentiating activity (in vitro and/or in vivo) is a major problem. With the expected emergence of numerous new differentiating agents, timely establishment of such screening systems seems appropriate.

Several problems have impeded the clinical development of differentiating agents. First, the concentration of agents which induces differentiation in vitro may not be achievable without in vivo side effects. Second, the in vitro differentiation dose may be cytotoxic in vivo. In addition, the criteria for the distinction between the process of differentiation and selective cytotoxicity followed by proliferation of benign elements have not been established. These problems are faced when most "low-dose" cytotoxic antineoplastic agents (eg, cytarabine, cytidine analogs, aclarubicin, NMF, etc) are used as differentiatir ɜ agents. Third, in contrast to the use of conventional cytotoxic chemotherapeutic agents which have tumor cell death as the endpoint of efficacy, the objective of using differentiating agents is to convert a malignant proliferation to a benign growth. Therefore, for the accurate and precise measurement of treatment effects with the use of differentiating agents at a clinical level, assessment of response by conventional phase II response criteria seems inadequate and new biologic endpoints of efficacy are obviously necessary.

Available data have demonstrated that HMBA is a potent inducer for the differentiation of various tumor cells including murine and human leukemias. Preliminary data also suggest that the compound inhibits tumor induction and reduces oncogenicity. In addition, the agent is not cytotoxic at concentrations that induce differentiation. One major difficulty expected in evaluating HMBA for its in vivo efficacy is achieving the in vitro concentrations which induce differentiation (> 2 mM) for a prolonged period (> 50 hours). So far, plasma concentrations of up to 4 mM have been achieved in animals and were tolerable (16,35,36). Current phase I trials are evaluating the schedule of either 5- or 10-day continuous infusion. More prolonged schedules of administration are also being considered. The use of a nonparenteral route (eg, oral) is a possible alternative to prolonged parenteral administration, and its development is in progress.

For the projected phase II trials, assessment of response by changes in either genotypic or phenotypic tumor markers should be considered in conjunction with

traditional response criteria. Evaluation of tumor markers, such as specific cell-surface markers, should indicate whether HMBA has induced cells to differentiate and has led them on a course of "programmed" cell death. Therefore, initial phase II efficacy trials should be highly restricted to specific tumor types in which such genotypic or phenotypic markers are well-defined and can be determined without significant difficulty.

REFERENCES

1. SPORN MB, ROBERTS AB, and DRISCOLL JS. Principles of cancer biology: growth factors and differentiation. *In* Cancer: Principles and Practice of Oncology, 2nd ed (DeVita VT, Jr, Hellman S, and Rosenberg SA, eds). Philadelphia, J. B. Lippincott Co, 1985, pp 49–65.
2. BLOCH A. Induced cell differentiation in cancer therapy. Cancer Treat Rep 68:199–205, 1984.
3. MARKS PA, and RIFKIND RA. Differentiation modifiers. Cancer 54:2766–2769, 1984.
4. FRIEND C, SCHER W, HOLLAND JG, ET AL. Hemoglobin synthesis in murine virus-induced leukemic cells in vitro: stimulation of erythroid differentiation by dimethyl sulfoxide. Proc Natl Acad Sci USA 68:378–382, 1971.
5. TANAKA M, LEVY J, TERADA M, ET AL. Induction of erythroid differentiation in murine virus infected erythroleukemia cells by highly polar compounds. Proc Natl Acad Sci USA 72:1003–1006, 1975.
6. REUBEN RC, WIFE RL, BRESLOW R, ET AL. A new group of potent inducers of differentiation in murine erythroleukemia cells. Proc Natl Acad Sci USA 73:862–866, 1976.
7. FIBACH E, REUBEN RD, RIFKIND RA, ET AL. Effect of hexamethylene bisacetamide on the commitment to differentiation of murine erythroleukemia cells. Cancer Res 37:440–444, 1977.
8. REUBEN RC, KHANNA PL, GAZITT Y, ET AL. Inducers of erythroleukemic differentiation: relationship of structure to activity among planar-polar compounds. J Biol Chem 253:4214–4218, 1978.
9. MARKS PA, REUBEN R, EPNER E, ET AL. Induction of murine erythroleukemia cells to differentiate: a model for the detection of new anti-tumor drugs. Antibiot Chemother 23:33–41, 1978.
10. MARKS PA, and RIFKIND RA. Erythroleukemic differentiation. Annu Rev Biochem 47:419–448, 1978.
11. REUBEN RC. Studies on the mechanism of action of hexamethylene bisacetamide, a potent inducer of erythroleukemic differentiation. Biochim Biophys Acta 588:310–321, 1979.
12. REUBEN RC, RIFKIND RA, and MARKS PA. Chemically induced murine erythroleukemic differentiation. Biochim Biophys Acta 605: 325–346, 1980.
13. COLLINS SJ, BODNER A, TING R, ET AL. Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leukemia cells. Int J Cancer 25:213–218, 1980.
14. MOON R. Unpublished report to Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Apr 9, 1984.
15. Clinical Brochure. Hexamethylene bisacetamide (HMBA; NSC-95580). Bethesda, MD, National Cancer Institute, Aug 1984.
16. MATSUO T, IMAMURA T, FUJITA K, ET AL. Differentiation of murine erythroleukemia cells by hexamethylenebisamide analogues. Acta Haematol Jpn 47:926–937, 1984.
17. RIFKIND RA. Unpublished report to Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Mar 30, 1984.
18. PALFREY C, KIMHI Y, LITTAUER UZ, ET AL. Induction of differentiation in mouse neuroblastoma cells by hexamethylene bisacetamide. Biochem Biophys Res Commun 76:937–942, 1977.
19. RABSON AS, STERN R, TRALKA TS, ET AL. Hemamethylene bisacetamide induces morphologic changes and increased synthesis of procollagen in cell line from glioblastoma multiforme. Proc Natl Acad Sci USA 74:5060–5064, 1977.
20. JAKOB H, DUBOIS P, EISEN H, ET AL. Effects of hexamethylene bisacetamide on the differentiation of embryonic cancer cells. C R Acad Sci Paris (Series D) 286:109–111, 1978.
21. PAULIN D, PERREAU J, JAKOB H, ET AL. Tropomyosin synthesis accompanies formation of actin filaments in embryonal carcinoma cells induced to differentiate by hexamethylene bisacetamide. Proc Natl Acad Sci USA 76:1891–1895, 1979.
22. LEVER JE. Inducers of mammalian cell differentiation stimulate dome formation in a differentiated kidney epithelial cell line (MDCK). Proc Natl Acad Sci USA 76:1323–1327, 1979.
23. Unpublished report to Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Mar 20, 1985.
24. BODGEN AE, COBB WR, BREITMAN TR, ET AL. HL-60/ascites tumors: a transplantable human tumor model for in vivo testing of differentiation inducers (DI's). Proc Am Assoc Cancer Res 26:34, 1985.
25. WAXMAN S, SCHER W, and SCHER BM. Basic principles utilizing combination differentiation agents. *In* Detection and Prevention of Cancer (Nieburgs HE, ed). New York, Alan R. Liss, Inc. In press.
26. TERADA M, FRIED J, NUDEL U, ET AL. Transient inhibition of initiation of S-phase associated with dimethyl sulfoxide induction of murine erythroleukemia cells to erythroid differentiation. Proc Natl Acad Sci USA 74:248–252, 1977.
27. FRIEDMAN EA, and SCHILDKRAUT CL. Lengthening of the G1 phase is not strictly correlated with differentiation in Friend erythroleukemia cells. Proc Natl Acad Sci USA 75:3813–3817, 1978.
28. GAZITT Y, REUBEN RC, DEITCH AD, ET AL. Changes in cyclic adenosine 3':5'-monophosphate levels during induction of differentiation in murine erythroleukemia cells. Cancer Res 38:3779–3783, 1978.
29. LEVY J, TERADA M, RIFKIND RA, ET AL. Induction of erythroid differentiation by dimethylsulfoxide in cells infected with Friend virus: relationship to the cell cycle. Proc Natl Acad Sci USA 72:28–32, 1975.
30. FERRERO D, TARELLA C, GALLO E, ET AL. Terminal differentiation of the human promyelocytic leukemia cell line, HL-60, in the absence of cell proliferation. Cancer Res 42:4421–4426, 1982.
31. SHEFFERY M, RIFKIND RA, and MARKS PA. Hexamethylene-bisacetamide-resistant murine erythroleukemia cells have altered patterns of inducer-mediated chromatin changes. Proc Natl Acad Sci USA 80:3349–3353, 1983.
32. PROFOU-JUCHELKA HR, REUBEN RC, MARKS PA, ET AL. Transcriptional and post-transcriptional regulation of globin gene accumulation in induced murine erythroleukemia cells. Mol Cell Biochem 3:229–232, 1983.
33. PAGE JG, BENEKE MB, and TOFT JD, II. Preclinical toxicology evaluation of HMBA (NSC-95580) in Beagle dogs and Sprague-Dawley rats. Technical report to the Toxicology Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Apr 27, 1984 (Documents PB84 192269 and PB84 192277, National Technical Information Service).
34. LITTERST CL, ROTH JS, and KELLEY JA. Distribution, elimination, metabolism and bioavailability of hexamethylenebisacetamide in rats. Invest New Drug 3:263–272, 1985.
35. KELLEY JA, ROTH JS, and LITTERST CL. Gas chromatographic determination of hexamethylene biacetamide in plasma and urine. Anal Lett 18(B9):1043–1062, 1985.

Synthesis, Physical Properties, and Antitumor Activity of Tetraplatin and Related Tetrachloroplatinum(IV) Stereoisomers of 1,2-Diaminocyclohexane[1,2]

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The synthesis, physical properties, and antitumor activity of the *cis-*, *d,l-trans-*, *d-trans-*, and *l-trans-* stereoisomers of 1,2-diaminocyclohexane tetrachloroplatinum(IV) are described. The objective of the study was to produce a platinum complex with activity against cisplatin-resistant tumor cells and with suitable pharmaceutical properties for formulation development. The isomers had the following solubilities in saline: *cis-*, 2 mg/ml; *d,l-trans-*, 6.5 mg/ml; and *d-trans-* and *l-trans-*, 15–16 mg/ml. The four complexes showed slightly better activity than cisplatin against the ip implanted murine L1210 leukemia. In contrast to cisplatin, all complexes produced significant increases in life span against L1210/cisplatin, a subline of L1210 with acquired resistance to cisplatin. However, the *cis-* isomer was less active against L1210/cisplatin. The *d,l-trans-* isomer (tetraplatin) was selected for further studies based on greater ease for large-scale synthesis. It showed superior activity to cisplatin against P388/cisplatin and like cisplatin showed significant and reproducible activity against the ip implanted B16 melanoma, ip implanted M5076 sarcoma, ip implanted P388 leukemia, and MX-1 human breast xenograft implanted under the renal capsule. Purity and stability (> 24 hours in saline) were evaluated by high-performance liquid chromatography and found to be suitable for development of a parenteral dosage form. Preliminary studies in a rat model (to be reported elsewhere) showed it to be less nephrotoxic than cisplatin on a molar basis and worthy of further study. [Cancer Treat Rep 70:997–1002, 1986]

Platinum analog development has been a fertile area of investigation based on the documented clinical efficacy of cisplatin either alone or in combination in the treatment of testicular, ovarian, bladder, and other cancers (1–3). However, the observation that cisplatin has several undesirable side effects (nephrotoxicity, ototoxicity, myelosuppression, neurotoxicity, severe nausea and vomiting, etc) as well as activity in only a limited number of tumor types has stimulated the synthesis and evaluation of many new agents in search of complexes with reduced toxicity and different biological properties (4–7). The discovery of platinum complexes with a different spectrum of antitumor activity has been made possible with the development of sublines of murine leukemias with acquired resistance to cisplatin (8,9). We wish to report the development of tetraplatin (fig 1), a platinum complex with activity against sublines of murine L1210 and P388 leukemias with acquired resistance to cisplatin, suitable pharmaceutical properties for formulation development, and reduced renal toxicity in rats compared to cisplatin (10,11).[6] This study extends earlier work on tetrachloroplatinum(IV) complexes of 1,2-diaminocyclohexane (1,2-DACH), since the previous reports raised questions concerning the purity, solubility, and activity of these complexes (12,13).

MATERIALS AND METHODS

Chemistry

Dichloroplatinum(II) 1,2-DACH complexes were prepared by treatment of equimolar concentrations of stereochemically defined isomers of *cis-*, *d,l-trans-*, and *d-trans-* or *l-trans-*1,2-DACH with potassium tetrachloroplatinate in *degassed* water under a nitrogen atmosphere (14,15). Each complex was purified by washing successively with 1 N HCl, ethanol, and diethyl ether. The sol-

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[6] Preliminary report of this work has appeared in Proc Am Assoc Cancer Res 26:261, 1985.
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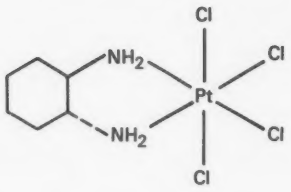

Tetraplatin, NSC 363812

Tetrachloro (*d, l-trans*) 1,2-diaminocyclohexane Pt (IV)

FIGURE 1.—Structure of tetraplatin (molecular weight = 451).

ids were dissolved in *N,N*-dimethylformamide (DMF), and then the solutions were filtered and treated with methanol to precipitate the complexes. Each complex was suspended in 0.5 N HCl and converted to the corresponding tetrachloroplatinum(IV) complex by treatment with chlorine gas at 100°C. Excess chlorine was then purged from each solution, solvent was removed, and the residues were dissolved in methanol. The methanol solutions were filtered and concentrated to dryness in vacuo and yellow crystalline products were obtained by crystallization from either dilute HCl or methanol.

The composition of each 1,2-DACH complex was verified by spectral data [^{13}C-nuclear magnetic resonance (NMR), ^{195}Pt-NMR, ^{1}H-NMR, FT-IR, and UV] and C, H, N, and Cl (± 0.3%) elemental analysis (Atlantic Microlabs, Atlanta, GA). A Varian FT-80 NMR spectrometer was used for the ^{13}C and ^{1}H spectra using D[7]-DMF [containing about 1% tetramethylsilane (TMS) as internal standard] as the solvent. The ^{195}Pt spectra were determined for DMF solutions using a Bruker 270-MHz NMR. IR spectra were determined for KBr pellets using a Nicolet FT-IR spectrophotometer and UV spectra for aqueous solutions were determined with a Cary 118 UV-vis spectrophotometer. Purity and stability were evaluated by high-performance liquid chromatography (HPLC) using a DuPont Zorbax C8 column with a 0.0075 M aqueous potassium phosphate buffer (pH = 4.9) as the mobile phase and 6-methyluracil as an internal standard. Additional purity and stability determinations were performed on tetraplatin using a DuPont Zorbax C8 column with H_2O as the mobile phase and thymine as an internal standard.

Biology

The antitumor activity of the complexes was evaluated according to standard protocols used by the Developmental Therapeutics Program, National Cancer Institute (NCI), Bethesda, MD (16,17). L1210/cisplatin, a line with acquired resistance to cisplatin, was developed by the late Dr. Frank M. Schabel, Jr, Southern Research Institute, Birmingham, AL. Mice bearing L1210 leukemia were treated with cisplatin at each passage until a stable, resistant line was developed in the absence of drug. A P388 line with acquired resistance to cisplatin (P388/cisplatin) was obtained from Dr. Joseph Burchenal, Memorial Sloan-Kettering Institute, New York, NY. Mice and other tumor lines were obtained through the Animal Genetics and Production Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, and were free of bacterial and viral contaminants as determined by routine microbiologic and murine antibody production testing. Lethal dose levels were determined for cisplatin and tetraplatin in nontumored male $CD2F_1$ mice (eight per dose level; treatment schedule was every 4th day for 3 days). Both agents were prepared fresh daily in saline. The LD10, LD50, and LD90 values for cisplatin were 11, 13.5, and 17 mg/kg, respectively. The corresponding values for tetraplatin were 10, 13.8, and 19 mg/kg.

RESULTS

Chemistry

The platinum complexes were each synthesized from a stereochemically defined isomer of 1,2-DACH as described in the Materials and Methods section and the overall yield of each (two steps) was about 83%. Thus, the final products maintained the stereoisomerism of the corresponding 1,2-DACH starting material (ie, the two NH_2 groups attached to the cyclohexane ring were in a *cis-*, *d-trans-*, or *l-trans-* position). The *d,l-trans-*complex (fig 1) was a mixture of equal amounts of *d-trans-* and *l-trans-*1,2-DACH.[7]

Spectral data and C, H, N, and Cl combustion analyses were consistent with the proposed structures of the four stereoisomers (*cis-*, *d,l-trans-*, *d-trans-*, and *l-trans-*). For simplicity, only the structure of the *d,l-trans-*isomer (tetraplatin) is shown (fig 1). All complexes were pure by HPLC analysis. The solubility of each isomer was determined in 0.9% saline, the preferred vehicle, and was found to be greater than for cisplatin (1 mg/ml): tetraplatin (6 mg/ml), *l-trans-* (15–16 mg/ml), *d-trans-* (15–16 mg/ml), and *cis-* (2 mg/ml). It is interesting to note that the *d,l-trans-* isomer (tetraplatin) is less soluble than either of the pure enantiomers. This difference presumably arises because of the different crystal forms for the individual enantiomers compared to the *d,l* mixture. Thus, the preparation of separate isomers resulted in products with enhanced solubility. By contrast, a mixture of *trans-* and *cis-*tetrachloroplatinum(IV) 1,2-DACH isomers showed a solubility of about 2 mg/ml (13), which is similar to the *cis-* isomer alone. A mixture of the *trans-* and *cis-*dichloroplatinum(II) 1,2-DACH isomers showed a solubility < 1 mg/ml (8) and was insoluble at the optimal dose. Although separation of the *trans-* and *cis-*1,2-DACH platinum(II) isomer resulted in products with different solubilities, none had a solubility > 1 mg/ml (15). An

[7] By contrast, *cis-* and *trans-*diamminedichloroplatinum are geometric isomers in which the –Cl and $-NH_3$ ligands are either *cis-* or *trans-* on the square planar complex.

attempt to improve the solubilities of the 1,2-DACH platinum(IV) tetrachloro isomers by making the corresponding dichlorodihydroxy derivative failed, since the dihydroxy derivatives were less soluble and were not soluble at optimal doses. Unpredictable solubility relationships involving other platinum(II) and platinum(IV) complexes have been previously noted (18). In regard to stability, tetraplatin showed < 10% decomposition in 0.9% saline at 25°C over a 24-hour period and therefore has a T90 > 24 hours. The corresponding value for the *cis*-1,2-DACH platinum(IV) isomer was 18 hours.

Antitumor Activity of the Stereoisomers

The antitumor activity of the four 1,2-DACH tetrachloroplatinum(IV) complexes and cisplatin was compared as shown in table 1. Each complex was administered by the ip route against the ip implanted L1210 and the L1210/cisplatin leukemias in a 60-day experiment. These data, as well as similar L1210 data from 30-day experiments (not shown), indicate that all four of the 1,2-DACH complexes are approximately equipotent with cisplatin on a milligram per kilogram basis. However, more 60-day survivors were found in the groups treated with the 1,2-DACH tetrachloroplatinum(IV) complexes than with cisplatin. Against L1210/cisplatin, the platinum(IV) complexes of the *trans*- isomers of 1,2-DACH were more efficacious than the corresponding *cis*-1,2-DACH isomer, and cisplatin was not effective as expected. The 1,2-DACH tetrachloroplatinum(IV) complexes also were evaluated by iv administration to mice bearing sc implanted L1210 leukemia. The complexes showed moderate activity (ILSs in the range of 25%–50%) comparable to that of cisplatin, and no problems were associated with the iv dosing (data shown only for tetraplatin in table 2). Based on its reduced activity against L1210/cisplatin and its lower water solubility, further development of the *cis*- isomer did not seem warranted. Likewise, separation of the *cis*- from the *trans*- isomers appeared important. Since only minor

TABLE 1.—Effect of cisplatin and the tetrachloroplatinum(IV) complexes of the stereoisomers of 1,2-DACH on the survival of mice bearing ip implanted L1210 leukemias sensitive and resistant to cisplatin*

Compound	Dose (mg/kg)	Ip L1210		Ip L1210/cisplatin	
		Body weight difference (g)	% ILS (60-day survivors)	Body weight difference (g)	% ILS (60-day survivors)
Cisplatin	20	−5.8		−5.6	
	10	−5.1	7	−5.1	
	5	−2.4	140	−2.3	10
	2.5	−1.4	54	−0.3	5
	1.25	−0.6	27	−0.1	3
l-trans-	16	−5.9		−7.3	
	8	−3.7	128 (1)	−3.5	33
	4	−2.2	108	−1.7	276 (3)
	2	−0.8	116 (2)	−0.8	139 (4)
	1	−0.4	46	−1.1	32
d,l-trans- (tetraplatin)	16	−5.9		−6.3	
	8	−3.2	78 (3)	−4.0	136
	4	−1.5	174	−1.2	351 (4)
	2	−1.2	80	−0.8	39 (1)
	1	−0.8	44	−0.6	29
d-trans-	16	−7.0		−6.1	
	8	−3.1	201 (3)	−4.8	93 (1)
	4	−1.2	102	−1.4	545 (6)
	2	−1.0	116 (3)	−0.2	47
	1	−0.6	44	−0.4	25
cis-	32	−5.6		−5.1	
	16	−5.6		−5.4	
	8	−2.3	213 (2)	−2.7	91 (1)
	4	−0.9	150 (2)	0.0	30
	2	−0.9	43	−0.3	21

*10^5 L1210 and L1210/cisplatin cells were implanted ip in $CD2F_1$ female mice on Day 0 according to previously published protocols (16,17). Drug treatments were given ip as single injections at the doses indicated on Days 1, 5, and 9 after tumor transplantation. Drugs were prepared fresh in 0.9% saline on the day of injection, and all were administered in solution. Body weights were determined on Day 5 and expressed as a weight difference (body weight of the drug-treated mice minus that of the control mice in grams). There were 10 mice/dose level. All mice, including 60-day survivors, were used in the calculation of median survival time (MST) and increase in life span (ILS). ILS = (100 × MST treated mice/MST control mice) − 100. The saline-treated control mice bearing L1210 leukemia lived a median of 8.3 days and gained 1.6 g by Day 5. The corresponding data for mice bearing L1210/cisplatin were 9.3 days and 1.3 g, respectively.

TABLE 2.—Antitumor activity of cisplatin and tetraplatin in direct comparison testing

Drug	Treatment schedule	Dose range (mg/kg)	Optimal dose (mg/kg)	Day of evaluation	% ILS*	TWI†	Score‡
			Ip B16 melanoma (murine tumor)				
Cisplatin	Ip daily × 9	0.12–2.00	2.0	60	90		++
Tetraplatin	Ip daily × 9	0.50–8.00	2.0	60	71		++
Cisplatin	Ip daily × 9	0.25–4.00	2.0	60	73		++
Tetraplatin	Ip daily × 9	0.25–4.00	1.0	60	30		+
Cisplatin	Ip daily × 9	0.50–2.00	2.0	60	147		++
Tetraplatin	Ip daily × 9	0.72–5.56	1.2	60	85		++
			Sc L1210 leukemia (murine tumor)				
Cisplatin	Iv daily × 5	1.25–10.0	5.0	30	38		+
Tetraplatin	Iv daily × 5	0.63–10.0	2.5	30	33		+
Cisplatin§	Ip daily × 9	0.25–4.0	2.0	30	54		++
Tetraplatin§	Ip daily × 9	0.33–10.7	2.7	30	81		++
			Ip M5076 sarcoma (murine tumor)				
Cisplatin	Ip on Days 1, 5, 9, and 13	0.25–4.0	4.0	60	133 (2/10)		++
Tetraplatin	Ip on Days 1, 5, 9, and 13	1.0–16.0	2.0	60	150 (3/10)		++
Cisplatin	Ip on Days 1, 5, 9, and 13	0.5–8.0	8.0	60	156 (3/10)		++
Tetraplatin	Ip on Days 1, 5, 9, and 13	0.5–8.0	4.0	60	147 (3/10)		++
			Ip P388 leukemia (murine tumor)				
Cisplatin	Ip on Days 1, 5, and 9	1.25–20.0	5.0	45	130		++
Tetraplatin	Ip on Days 1, 5, and 9	1.00–16.0	8.0	45	111		++
Cisplatin	Ip on Days 1, 5, and 9	2.30–10.7	10.7	45	190 (1/10)		++
Tetraplatin	Ip on Days 1, 5, and 9	2.30–10.7	5.3	45	125		++
			Ip P388/cisplatin leukemia (murine tumor)				
Cisplatin	Ip on Days 1, 5, and 9	1.25–20.0	10	45	11		−
Tetraplatin	Ip on Days 1, 5, and 9	1.00–16.0	8	45	98		++
Cisplatin	Ip on Days 1, 5, and 9	2.30–10.7	8	45	14		−
Tetraplatin	Ip on Days 1, 5, and 9	2.30–10.7	5.3	45	117		++
			Subrenal capsule MX-1 breast (human tumor)				
Cisplatin§	Ip on Days 1, 5, and 9	1.66–13.3	6.65	11		−34	++
Tetraplatin§	Ip on Days 1, 5, and 9	2.00–16.0	8.00	11		93	++
Cisplatin§	Ip on Days 1, 5, and 9	2.00–8.0	4.00	11		−92	++
Tetraplatin§	Ip on Days 1, 5, and 9	4.00–32.0	8.00	11		95	++

*All testing was conducted according to previously published protocols (16,17). All animals, including those alive on the final day of evaluation, were used in the calculations of ILS. Cures are defined as animals without gross evidence of tumor on the final day of evaluation. Values in parentheses = No. of cures/total No. of animals.

†Negative No. = tumor regression, and positive No. = tumor weight inhibition.

‡++ = ≥ 50% ILS for ip B16 melanoma, sc L1210 leukemia, and ip M5076 sarcoma; ≥ 75% ILS for ip P388 and ip P388/cisplatin leukemias; and > 90% tumor weight inhibition (TWI) for the subrenal capsule model; + = 25%–49% ILS for ip B16 melanoma, sc L1210 leukemia, and ip M5076 sarcoma; 20%–74% ILS for P388 and P388/cisplatin leukemias; and 80%–90% TWI for the subrenal capsule; − = inactive.

§Not a direct comparison.

differences were observed among the *trans-* isomers in regard to solubility, stability, and antitumor properties, the *d,l-trans-* isomer (tetraplatin) was selected for further study based on its ease of large-scale synthesis.

Additional Antitumor Studies on Tetraplatin

Tetraplatin was also examined against several other tumor systems. As summarized in table 2, there were no significant differences between cisplatin and tetraplatin in the spectrum of antitumor activity except in the two leukemias (L1210, table 1; and P388, table 2) with acquired resistance to cisplatin. In these latter two lines, cisplatin was devoid of activity, whereas tetraplatin produced increases in life span > 300% and four of ten 60-day survivors against L1210/cisplatin (table 1) and increases > 90% against P388/cisplatin (table 2). Significant and reproducible activity at optimal doses was observed for cisplatin and tetraplatin against the ip implanted B16 melanoma, ip implanted L1210 leukemia, sc implanted L1210 leukemia, ip implanted M5076 sarcoma, ip implanted P388 leukemia, and the human MX-1 breast tumor xenograft (table 2). Cisplatin showed slightly greater increases in life span than tetraplatin

against both the ip implanted B16 melanoma and P388 leukemia tumor systems in direct comparison tests. Against the human MX-1 breast tumor xenograft, cisplatin produced tumor regressions, whereas tetraplatin produced only growth inhibition at tolerated doses (< 4 g of weight loss); however, these comparisons were not conducted in the same experiment. To determine whether these slight differences are statistically significant would require considerable additional testing. Small quantitative differences among platinum complexes have been previously noted (19) and are of unknown basis or significance.

DISCUSSION

Tetraplatin represents a significant development in second-generation platinum complexes because of its solubility, stability, purity, ease of preparation, and spectrum of antineoplastic activity. Complexes based on 1,2-DACH were synthesized because of previous observations that platinum complexes with this substitution retain activity against sublines of L1210 and P388 leukemia with acquired resistance to cisplatin (8,9). However, previously described complexes lacked sufficient stability or solubility in aqueous medium for the development of iv preparations for wide-scale use (9,20–26). Tetraplatin is a 1,2-DACH complex with suitable pharmaceutical properties. The availability of stereochemically defined *d,l-trans*-1,2-DACH as starting material makes the large-scale synthesis of tetraplatin feasible. The development of a reliable stability-indicating assay (HPLC) assures batch-to-batch reproducibility. The platinum(IV) oxidation state was chosen for study because octahedral platinum(IV) complexes tend to be more soluble and stable than the corresponding square planar platinum(II) complexes (27). They also are thought to serve as prodrugs for the corresponding platinum(II) complexes (27). The basis for the slight differences among the *cis*- and *trans*- isomers in antitumor efficacy is not understood at this time but may relate to differences in cellular uptake or binding of the complexes to DNA, as previously postulated for 1,2-DACH platinum(II) isomers (15). One may argue that separation of the isomers is not important since only subtle differences in activity have been reported among other 1,2-DACH isomers (15) and that the antitumor results with tetraplatin are not that different from those reported for the diastereoisomeric mixture in L1210 (13) or for diastereoisomeric mixtures of other 1,2-DACH-containing platinum complexes in L1210/cisplatin (28). However, based on its lower solubility and stability and its reduced activity against L1210/cisplatin, the inclusion of the *cis*- isomer in the final product does not appear justified. With more detailed studies of the isomers, other differences (such as in binding to DNA) may become apparent, as have been demonstrated with other compounds (29–31).

The clinical relevance of the activity of tetraplatin in murine tumors with acquired resistance to cisplatin remains to be studied. Furthermore, the mechanisms of resistance to cisplatin in the murine systems used in this study and in the clinic have not been defined. It is accepted that drug resistance continues to be a major factor limiting the effectiveness of cancer chemotherapy, and that the design of second-generation analogs which retain their effect in the presence of a resistance mechanism warrants greater emphasis (32). Trump and Hortvet (33) have estimated that 10%–20% of patients with cancer of the testis die of disease which is refractory to treatment with cisplatin-based combination chemotherapy. Also, they and others have stated that improved therapy is needed for patients with poor prognosis and for patients failing to achieve a durable remission following initial therapy. Given the scope of the problem of drug resistance in the clinic and the observations that some murine lines respond to cisplatin but not cyclohexane-containing complexes and other lines respond to cyclohexane-containing complexes but not cisplatin (34), it appears reasonable that more than one type of platinum complex should be developed to the clinic. It is interesting to note that both carboplatin and iproplatin, two platinum complexes under active clinical investigation, lack activity against the L1210 and P388 sublines with acquired resistance to cisplatin (28).

Based on its spectrum of activity and its suitable pharmaceutical properties, tetraplatin is being evaluated by the NCI as a potential clinical trial candidate. Preliminary studies in a rat model showed it to be less nephrotoxic than cisplatin on a molar basis (10,11). More definitive toxicologic studies in the dog are planned.

REFERENCES

1. CARTER SK. Cisplatin—past, present and future. *In* Platinum Coordination Complexes in Cancer Chemotherapy (Hacker MP, Douple EB, and Krakoff IH, eds). Boston, Martinus Nijhoff Publishing, 1984, pp 359–376.
2. DURANT JR. Cisplatin: a clinical overview. *In* Cisplatin, Current Status and New Developments (Prestayko AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 317–321.
3. LOEHRER PJ, and EINHORN LH. Drugs five years later. Cisplatin. Ann Intern Med 100:704–713, 1984.
4. CLEARE MJ, HYDES PC, HEPBURN DR, ET AL. Antitumor platinum complexes: structure-activity relationships. *In* Cisplatin, Current Status and New Developments (Prestayko AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 149–170.
5. CONNORS TA, CLEARE MJ, and HARRAP KR. Structure-activity relationships of the antitumor platinum coordination complexes. Cancer Treat Rep 63:1499–1502, 1979.
6. HARRAP KR. Platinum analogs: criteria for selection. *In* Cancer Chemotherapy, Vol 1 (Muggia FM, ed). Boston, Martinus Nijhoff Publishing, 1983, pp 171–217.
7. HYDES PC. Synthesis and testing of platinum analogues—an overview. *In* Platinum Coordination Complexes in Cancer Chemotherapy (Hacker MP, Douple EB, and Krakoff IH, eds). Boston, Martinus Nijhoff Publishing, 1984, pp 216–227.
8. BURCHENAL JH, KALAHER K, DEW K, ET AL. Rationale for development of platinum analogs. Cancer Treat Rep 63:1493–1498, 1979.
9. BURCHENAL JH, IRANI G, KERN K, ET AL. 1,2-Diaminocyclohexane platinum derivatives of potential clinical value. Recent Results Cancer Res 74:146–155, 1980.
10. SMITH JH, SMITH MA, LITTERST C, ET AL. Nephrotoxicity of

tetraplatin: an alternative to cisplatin. Pharmacologist 27:199, 1985.
11. SMITH MA, SMITH JH, LITTERST C, ET AL. Early noninvasive detection of tetraplatin and cisplatin nephrotoxicity. Pharmacologist 27:199, 1985.
12. HALL LM, SPEER RJ, RIDGWAY HJ, ET AL. Analogs of sulfato-1,2-diaminocyclohexane platinum (II) SHP II. Modifications other than leaving ligand. Wad Med Bull 7:231-241, 1977.
13. SCHWARTZ P, MEISCHEN SJ, GALE GR, ET AL. Preparation and antitumor evaluation of water-soluble derivatives of dichloro(1,2-diaminocyclohexane)platinum(II). Cancer Treat Rep 61:1519-1525, 1977.
14. KIDANI Y, and SAITO R. Preparative separation of cis- and trans-1,2-diaminocyclohexane mixture by means of the selective nickel (II) complex formation. Chem Lett 123-126, 1976.
15. KIDANI Y, INAGAKI K, IIGO M, ET AL. Antitumor activity of 1,2-diaminocyclohexane-platinum complexes against Sarcoma-180 ascites form. J Med Chem 21:1315-1318, 1978.
16. GERAN RI. GREENBERG NH, MACDONALD MM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (3rd ed). Cancer Chemother Rep Part 3, vol 3(2):1-103, 1972.
17. In Vivo Cancer Models 1976-1982, NIH Publication No. 84-2635, Feb 1984.
18. TOBE ML, and KHOKHAR AR. Structure, activity, reactivity and solubility relationships of platinum diamine complexes. Wad Med Bull 7:114-137, 1977.
19. WOLPERT-DEFILIPPES MK. Antitumor activity of cisplatin analogs. *In* Cisplatin, Current Status and New Developments (Prestayko AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 183-191.
20. CONNORS TA, JONES M, ROSS WCJ, ET AL. New platinum complexes with antitumor activity. Chem Biol Interact 5:415-424, 1972.
21. FARRELL NP, HACKER MP, KHOKHAR AR, ET AL. Chemical characterization of water soluble *N*-substituted iminodiacetato-(1,2-diaminocyclohexane)-platinum (II) antitumor complexes. Proc Am Assoc Cancer Res 26:263, 1985.
22. HACKER MP, KHOKHAR AR, BROWN DB, ET AL. Ascorbato (1,2-diaminocyclohexane): platinum (II) complexes, a new series of water-soluble antitumor drugs. Cancer Res 45:4748-4753, 1985.
23. HOLLIS SL, AMUNDSEN AR, and STERN EW. Synthesis, structure and antitumor properties of the platinum complexes of vitamin C. J Am Chem Soc 107:274-276, 1984.
24. KELSON DP, SCHER H, and BURCHENAL J. Phase I and early Phase II trials of 4'-carboxyphthalato (1,2-diaminocyclohexane) platinum (II). *In* Platinum Coordination Complexes in Cancer Chemotherapy (Hacker MP, Douple EB, and Krakoff IH, eds). Boston, Martinus Nijhoff Publishing, 1984, pp 310-320.
25. MATHÉ G, KIDANI Y, MARAL R, ET AL. Experimental study of oxalato-platinum complex of *trans*-1-diaminocyclohexane (1-OHP) in murine tumors. Early Phase I study in man. Proc Am Assoc Cancer Res 26:264, 1985.
26. MACQUET JP, CROS S, and ARMAND JP. Pharmacological and preclinical toxicological studies on 1,2-diaminocyclohexane (isocitrato) platinum (II). Cancer Res 44:3736-3743, 1984.
27. DROBNIK J. Antitumor activity of platinum complexes. Cancer Chemother Pharmacol 10:145-149, 1983.
28. ROSE WC, and BRADNER WT. Experimental antitumor activity of platinum coordination complexes. *In* Platinum Coordination Complexes in Cancer Chemotherapy (Hacker MP, Douple EB, and Krakoff IH, eds). Boston, Martinus Nijhoff Publishing, 1984, pp 228-239.
29. SHERMAN SE, GIBSON D, WANG AHJ, ET AL. X-ray structure of the major adduct of the anticancer drug cisplatin with DNA: *cis*-[$Pt(NH_3)_2$ {d(pGpG)}]. Science 230:412-417, 1985.
30. ARIENS EJ. Domestication of chemistry by design of safer chemicals: structure-activity relationships. Drug Metab Rev 15:425-504, 1984.
31. GEUENS GMA, NUYDENS RM, WILLEBRORDS RD, ET AL. Effects of tubulozole on the microtubule system of cells in culture and in vivo. Cancer Res 45:733-742, 1985.
32. GOLDIE JH, and COLDMAN AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. Cancer Res 44:3643-3653, 1984.
33. TRUMP DL, and HORTVET L. Etoposide and very high dose cisplatin: salvage therapy for patients with advanced germ cell neoplasms. Cancer Treat Rep 69:259-261, 1985.
34. EASTMAN A, and ILLENYE S. Murine leukemia L1210 cell lines with different patterns of resistance to platinum coordination complexes. Cancer Treat Rep 68:1189-1190, 1984.

Role of Hexamethylmelamine in the Treatment of Ovarian Cancer: Where Is the Needle in the Haystack?[1]

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Hexamethylmelamine (HMM) was selected for development as an antineoplastic agent because it demonstrated activity in a variety of preclinical tumor models. Its mechanism of action is unknown. It has been used in clinical trials since 1964. The clinical toxic effects have consisted of signs and symptoms involving the following systems: gastrointestinal (nausea, vomiting, anorexia), hematologic (leukopenia, mild anemia), and neurologic (critical depression, hallucinations, peripheral motor and sensory deficits). Antitumor activity against advanced ovarian cancer was demonstrated in phase I trials and the drug was quickly incorporated into trials which utilized drug combinations. The majority of these have consisted of phase II trials without an identified control population. As might be predicted, all of the HMM-containing combinations are active. However, the contribution of HMM to the antitumor activity of the combination remains conjectural. Thus, in spite of > 15 years of clinical trials with a drug that has single-agent activity, the questions regarding the role of HMM in the treatment of ovarian cancer remain unanswered. [Cancer Treat Rep 70:1003–1014, 1986]

Hexamethylmelamine (HMM), an *S*-triazene, is a substituted melamine derived from cyanuric chloride (1). Although it has structural similarities to triethylenemelamine (fig 1), a classical alkylating agent, the mechanism of action of HMM is unknown. The species thought to be responsible for the antitumor activity is a product(s) from the metabolism of the drug through oxidative *N*-demethylation (2).

The best preclinical antitumor activity was against the Walker 256 rat carcinosarcoma (3). HMM has also shown activity against the $CD8F_1$ mammary tumor and the Lewis lung carcinoma (4). In nude mice it has shown activity against human xenografts of mammary (4), lung (4), pancreatic (5), and colon and rectal carcinoma (6). In the subrenal capsule model, activity was demonstrated against a mammary tumor (4).

In this paper we review the human pharmacology, the phase I trials, and the results of clinical trials for the treatment of patients with advanced-stage ovarian cancer. Although the oncologic literature is replete with results of trials from HMM-containing combinations, the Discussion section describes the difficulties encountered when an attempt is made to define the role of HMM in the treatment of this disease.

In summarizing the results of these many clinical trials, the authors' response rates, survival figures, and statistical analyses have been cited directly. Response rates are based on "evaluable" patients; unfortunately, evaluability criteria are variable. It was not possible from the information furnished in the publications to re-evaluate response in a uniform manner. Confidence intervals for response rates were calculated by us using exact formulas for the binomial distribution. Confidence intervals for the difference in two response rates were calculated by us in selected instances using the usual normal distribution approximation. All confidence intervals are the two-sided type. The statistical power of the Gynecological Oncology Group (GOG) trial (47,48) for detecting differences in survival was calculated by us based upon the reported number of deaths per treatment using formulae for the exponential survival distribution.

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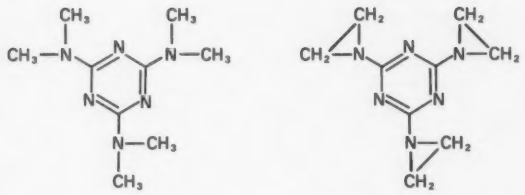

HEXAMETHYLMELAMINE TRIETHYLENEMELAMINE

FIGURE 1.—Structures of hexamethylmelamine and triethylenemelamine.

Human Pharmacology

Because of the difficulties encountered with the administration of a parenteral form of HMM, the clinical trials reported here have used only the oral form. Results from earlier human pharmacokinetic studies were reported by Bryan et al (7) and Worzalla et al (8,9), who used ^{14}C radioisotope-labeled drug at either the triazine ring or one of the methyl groups. The peak plasma level of radioactivity occurred at 1 hour, with a plasma half-life of 13 hours (7). HMM is primarily metabolized by *N*-demethylation in liver microsomes, which results in the sequential removal of methyl groups that are oxidized to CO_2 (8). The remaining triazine ring is excreted intact in the urine (7). When ^{14}C ring-labeled HMM was used, 61% of the label was secreted in the urine in 24 hours and 89% by 72 hours (9). More recent studies by D'Incalci et al (10), using a gas chromatographic assay, showed that peak plasma levels were obtained in 1–3 hours (range, 0.5–20), with a plasma half-life of 4.6–10.2 hours. The patients received their drug either in a fasting state or before meals over the dose range of 140–300 mg/m^2/day and there was considerable variability in drug bioavailability and metabolism (10).

Phase I Studies

A phase I study reported by Wilson and de la Garza (11) evaluated 49 patients using the micronized oral preparation and reached a maximum tolerated dose of 10–12 mg/kg/day for 21 days. Louis et al (12) reported another phase I study where 124 patients were evaluated, and some were treated with HMM for up to 214 days. The dose tolerated by 80% of the patients was 10 mg/kg/day for 28 days. In both studies, the dose-limiting toxic effects were gastrointestinal (anorexia, nausea, vomiting, abdominal cramps, diarrhea), weight loss, and hematologic (anemia, thrombocytopenia, or leukopenia). When patients were treated for prolonged time periods (> 28 consecutive days), there were reports of peripheral neuropathy. The neuropathy consisted of paresthesias, hypesthesias, hyporeflexia, motor weakness, decreased position and vibratory sense, and decreased sensory and proprioceptive sensations. The symptoms usually resolved after the discontinuation of HMM (12). Antitumor activity was reported in patients with ovarian, lung, breast, and prostatic carcinoma (11).

Ovarian Cancer Trials With HMM

Single-Agent Trials

The responses observed during the phase I trials in the early 1970s led to several phase II trials (table 1) in patients with advanced ovarian cancer[5] (13–21). (Untreated patients usually die of disease within 6–12 months of diagnosis.) HMM was usually given at doses of 8 mg/kg/day for variable periods of 14–21 days in monthly cycles or until toxicity. Daily continual administration up to 90 days did not increase the objective

[5] Advanced disease is defined as stage III or IV by the International Federation of Gynecology and Obstetrics.

TABLE 1.—Trials of single-agent HMM in patients with advanced or refractory ovarian carcinoma*

Group or institution (ref No.)	Dose schedule	No. of evaluable patients	No. of responses			80% confidence interval for CR + PR (%)
			CR	Total	PR + CR (%)	
M. D. Anderson (13,14)	6–8 mg/kg/day until toxicity	54	8	17	31.5	23–41
Central Oncology Group (15)	12 mg/kg/day for 21 days	12	None	5	42	22–63
Central Oncology Group (16)	8 mg/kg/day for 90 days	10	1	3	33	12–55
Rush-Presbyterian-St. Lukes (17)	250 mg/m^2/day until toxicity	16	1	4	25	11–44
NCI Medicine Branch (18)	8 mg/kg/day	21	None	4	19	9–35
Southeastern Cancer Study Group (19)	280 mg/m^2/day until toxicity	49	1	8	16	10–25
M. D. Anderson (20)	8 mg/kg/day	12		1	8.3	1–29
Milan (21)	6 mg/kg/day for 21 days every 4 wks or 8 mg/kg/day for 21 days every 6 wks	34	None	2	6	2–15

*CR = complete response; PR = partial response.

response rate in a small series of patients (16). Instead, the incidence of side effects, especially neurological toxic effects, was aggravated. The highest response rates (31%–42%) were reported in patients who were either chemotherapy-naive or had not been treated with an alkylating agent (13–16). Response rates observed near 20% could still be obtained in spite of prior alkylator therapy, provided progressive disease had been demonstrated to only *one* agent (17–19). However, when two or more agents were used in the prior chemotherapy treatment, the response rates declined to ≤ 10% (20,21).

The most important factor associated with increased survival in patients with advanced ovarian cancer is a complete response that has been documented by surgical re-exploration with biopsies of the previous sites of disease, biopsies of areas that are common sites of residual ovarian cancer, and saline washings of the abdomen. All of the pathological samples must be negative (pathologically documented complete response). Even in the trials with the most favorable patient populations treated with single-agent HMM (13–16), there were few complete responses. In the largest series of patients treated with single-agent HMM as their first chemotherapy, the complete response rate was only 15% (13,14). However, the patients did not consistently have surgical restaging performed; thus, pathologically documented complete responses were not delineated. The lack of a significant increase in the median survival reported from this study suggests that HMM did not produce many pathological complete responses for patients with advanced disease.

Single agents frequently used during the 1970s, eg, alkylators, methotrexate, 5-FU, or doxorubicin, were compared to HMM in prospectively randomized phase III studies; these agents produced objective response rates of 30%–39%, except for 5-FU, which produced a response rate of 12% (22). The large variability among known prognostic factors, including stage of disease (23), tumor burden (24), histologic grade (25), and prior chemotherapy (20), precludes reliable cross-study comparisons. However, most of the published information from the early to mid-1970s suggests that alkylators were the agents most frequently used for single-agent therapy (26).

HMM-Containing Combination Studies

Since the 5-year survival rate for advanced-disease patients remained unaffected by single-agent chemotherapy, interest in exploring the potential that combination chemotherapy might produce higher response rates and improved survival gave rise to a large number of HMM-containing combination chemotherapy trials. Many of these trials combined HMM with alkylators and other agents that had shown single-agent activity, including cisplatin (tables 2–4).

Table 2 contains published results of HMM-based regimens in patients who had received chemotherapy prior to receiving the HMM-containing combination (27–45). In the great majority of these trials, so few patients were entered that the confidence intervals are too broad to allow conclusions based on comparisons between studies.

For the nonrandomized trials of combinations containing both HMM and cisplatin in the treatment of patients who had been previously treated with chemotherapy, the response rates were quite variable (33–45). However, as with the single-agent results, long-term complete responders were rare and the median survival times cluster around 10 months, irrespective of the specific agents used in the combination.

Only two of the 19 trial designs might have defined the specific role of HMM in the treatment of patients with this disease (44,45). In the first, HMM-doxorubicin-cisplatin was compared with cyclophosphamide-doxo-

TABLE 2.—Trials with combination regimens containing HMM in patients with advanced ovarian carcinoma who had failed treatment with prior chemotherapy*

Group or institution (ref No.)	Regimen	Interval	No. of evaluable patients	No. of responses			Median survival (mos)	80% confidence interval for CR + PR (%)
				CR	Total	CR + PR (%)		
University of Nebraska (27)	HMM 50–150 mg/m^2 daily × 14 Melphalan 1–7 mg/m^2 daily × 5	Every 28 days	10	2	2	20	22	5–45
M. D. Anderson Hospital (28)	HMM 4 mg/kg daily × 14 CTX 250 mg/m^2 daily × 5 (oral)	Every 28 days	30	1	4	13	10.5	6–25
Western Cancer Study Group (29)	HMM 8 mg/kg Days 1–21 5-FU 12 mg/kg weekly × 4	Every 6 wks	14	3	5	36	9	18–56
Milan (30)	HMM 150 mg/m^2 Days 1–14 DOX 50 mg/m^2 Day 1 CTX 100 mg/m^2 Days 1–14 (oral)	Every 28 days	30	1	5	16	10.5	8–29
University Hospital, Boston (31)	HMM 300 mg/m^2 Days 8–21 Cisplatin 50 mg/m^2 Day 1	Every 28 days	13	None	2	15	NR	4–36

continued

TABLE 2 (*continued*)

Group or institution (ref No.)	Regimen	Interval	No. of evaluable patients	No. of responses			Median survival (mos)	80% confidence interval for CR + PR (%)
				CR	Total	CR + PR (%)		
Albert Einstein, Montefiore (32,37)	HMM 200 mg/m^2 Days 8–21 Cisplatin 50 mg/m^2 Day 1	Every 21 days	5	1	4	80	NR	41–98
North Central Oncology Group (33)	HMM 150 mg/m^2 Days 2–11 Cisplatin 60 mg/m^2 Day 1	Every 28 days	28	None	4	14	NR	6–27
Eastern Cooperative Oncology Group (34)	HMM 200 mg/m^2 Days 8–21 Cisplatin 75–100 mg/m^2 Day 1	Every 21 days	38	9	21	55	10.8	44–66
Denmark (35)	HMM 200 mg/m^2 Days 8–21 Cisplatin 75 mg/m^2 Day 1	Every 28 days	29	2	10	34	NR	23–48
Southwestern Oncology Group (36)	HMM 150 mg/m^2 Days 1–14 Cisplatin 50 mg/m^2 Day 1 5-FU 400 mg/m^2 Days 1, 8	Every 28 days	74	None	23	31	14	24–39
Eastern Cooperative Oncology Group (32,37)	HMM 200 mg/m^2 Days 8–21 DOX 30 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 21 days	22	4	14	63	9	48–78
Central Pennsylvania Oncology Group (38)	HMM 150 mg/m^2 Days 1–14 DOX 40 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 CTX 300 mg/m^2 Day 1	Every 28 days	35	7	17	49	NR	37–61
Roswell Park (39)	HMM 150 mg/m^2 Days 8–21 DOX 25 mg/m^2 Day 1 Cisplatin 1 mg/kg Day 1 CTX 400 mg/m^2 Day 1	Every 28 days	20	None	None	0	NR	0–11
Southwest Oncology Group (36,40)	HMM 150 mg/m^2 Days 1–14 DOX 25 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 5-FU 300 mg/m^2 Days 1, 8	Every 28 days	29	2	14	48	12+	35–62
Vanderbilt University (41)	HMM 150 mg/m^2 Days 1–14 DOX 20 mg/m^2 Days 1, 8 Cisplatin 60 mg/m^2 Days 1, 8 5-FU 400 mg/m^2 Days 1, 8	Every 28 days	12	4	11	92	13	71–90
Mt. Sinai, NY (42)	HMM 150 mg/m^2 Days 2–7 DOX 30 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 CTX 150 mg/m^2 Days 2–7 (oral)	Every 28 days	21	3	10	48	7	32–60
Mt. Sinai, NY (43)	HMM 150 mg/m^2 Days 4–10 → 17 DOX 30–50 mg/m^2 Day 3 Cisplatin 50 mg/m^2 Day 1 CTX 500 mg/m^2 Day 3	Every 28 days	24	6	13	54	NR	39–60
Central Pennsylvania Oncology Group (44)	Ⓡ HMM 150 mg/m^2 Days 2–15 DOX 40 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 28 days	27	6	11	41	7.5	28–55
	Ⓡ CTX 300 mg/m^2 Day 1 DOX 40 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 28 days	23	3	7	30	13.5	18–46
Netherlands (45)	Ⓡ HMM 150 mg/m^2 Days 15–28 DOX 35 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 35 days	23	1	4	17	5.3	8–32
	Ⓡ DOX 35 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 21 days	22	1	5	23	6.9	12–38

*CR = complete response; PR = partial response; CTX = cyclophosphamide; DOX = doxorubicin; Ⓡ indicates that patients entered on this study were randomly assigned to treatment arms; and NR = not reported.

TABLE 3.—Trials with combination regimens containing HMM + alkylators in patients with advanced ovarian carcinoma who had not received chemotherapy*

Group or institution (ref No.)	Regimen	Interval	No. of evaluable patients	No. of responses			Median survival (mos)	80% confidence interval for CR + PR (%)
				CR	Total	CR + PR (%)		
Gynecologic Oncology Group (47,48)	HMM 100–150 mg/m^2 daily × 14 Melphalan 5–7 mg/m^2 daily × 5	Every 28 days	97	27	50	52	13.5	45–59
(R)	DOX 40–50 mg/m^2 Day 1 CTX 500 mg/m^2 Day 1	Every 21 days	72	23	45	63	14.2	54–70
	Melphalan 5–7 mg/m^2 daily × 5	Every 28 days	64	13	24	38	12.3	29–46
Georgetown University (49)	HMM 150 mg/m^2 Days 1–14 CTX 100 mg/m^2 Days 1–14 (oral) 5-FU 600 mg/m^2 Days 1–8	Every 28 days	13	7	11	85	NR	64–96
(R)	Melphalan 0.2 mg/kg daily × 5 (oral)	Every 28 days	14	3	8	57	NR	37–76
NCI Medicine Branch (50)	HMM 150 mg/m^2 Days 1–14 CTX 100 mg/m^2 Days 1–14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 600 mg/m^2 Days 1, 8	Every 28 days	40	13	30	75	29	64–84
(R)	Melphalan 0.2 mg/kg daily × 5 every 28–35 days		37	6	20	54	17	42–66
Portugal (51)	HMM 150 mg/m^2 Days 1–14 CTX 150 mg/m^2 Days 1–14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 600 mg/m^2 Days 1, 8	Every 28 days	28	4	10	36	10	24–50
(R)	CTX 40 mg/kg Day 1 every 3 wks		29	7	18	62	11	49–74
Milan (52)	HMM 150 mg/m^2 Days 1–14 DOX 50 mg/m^2 Day 1 CTX 70 mg/m^2 Days 1–14	Every 28 days	49	14	31	63	23	53–73
(R)	Cisplatin 50 mg/m^2 Day 1 DOX 50 mg/m^2 Day 1 CTX 70 mg/m^2 Days 1–14 (oral)	Every 28 days	54	18	36	67	24	67–75
Yale University (53)	HMM 160 mg/m^2 Days 1–14 CTX 250 mg/m^2 Days 1–5 (oral)	Every 28 days	20	6	10	50	13.7	34–66
(R)	DOX 40 mg/m^2 Day 1 CTX 250 mg/m^2 Day 1 (oral)	Every 28 days	17	6	10	59	16.7	41–75
Princess Margaret (54)	HMM 150 mg/m^2 Days 1–14 CTX 150 mg/m^2 Days 1–14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 600 mg/m^2 Days 1, 8	Every 28 days	37	7	NR	NR	NR	
(R)	CTX 500 mg/m^2 Day 1 DOX 50 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 21 days	40	12	NR	NR	NR	
	Melphalan 8 mg/m^2 daily × 5		38	5	NR	NR	NR	
M. D. Anderson Hospital (55)	HMM 4 mg/kg Days 1–14 DOX 40 mg/m^2 Day 1 CTX 200 mg/m^2 Days 1–5 (oral)	Every 28 days	71	20	22	31	26.4	24–39
(R)	Melphalan 0.2 mg/m^2 Days 1–5 Cisplatin 20 mg/m^2 Days 2–4	Every 28 days	82	27	31	38	29.6	31–46

continued

TABLE 3 (*continued*)

Group or institution (ref No.)	Regimen	Interval	No. of evaluable patients	No. of responses			Median survival (mos)	80% confidence interval for CR + PR (%)
				CR	Total	CR + PR (%)		
Netherlands (56)	HMM 150 mg/m^2 Days 1–14 CTX 150 mg/m^2 Days 1–14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 600 mg/m^2 Days 1, 8	Every 28 days	23	5	12	52	NR	37–67
Milan (57)	HMM 100 mg/m^2 Days 1–14 CTX 100 mg/m^2 Days 1–14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 600 mg/m^2 Days 1, 8	Every 28 days	31	7	13	42	16.5	30–55

*MTX = methotrexate; other abbreviations as in footnote to table 2.

rubicin-cisplatin, while in the second trial, HMM-doxorubicin-cisplatin was compared to a combination of doxorubicin and cisplatin. Although there were no significant differences observed in response rates or survival on the arms of these two randomized studies, the number of patients randomized in each trial is insufficient to distinguish true differences of less than about 40% in response rates.

Combination chemotherapy, as a front-line approach, has sometimes been associated with higher response rates than single-agent chemotherapy (46). However, the long-term efficacy of combination drug regimens (tables 3 and 4) compared to single agents remains controversial (47–65). HMM was contained in the regimen used in the first randomized trial that reported superiority of combination chemotherapy over single agents (50). Since then, HMM-containing combinations have been used extensively in the treatment of patients with advanced ovarian cancer (tables 3 and 4).

Of the non-cisplatin-containing HMM combinations listed in table 3 (47–57), the highest response rate from studies that reported on at least 20 patients was with Hexa-CAF (HMM, cyclophosphamide, 5-FU, and methotrexate), as reported by the Medicine Branch of the National Cancer Institute (NCI) (50). They reported an objective response rate of 75% among 40 evaluable patients, with 32% clinical complete responses. The median of the observed response rates from all combination studies listed in table 3 was 46.5%, while the median for all the cisplatin-containing HMM combinations (table 4) was 63%.

DISCUSSION

The most active drugs against advanced ovarian cancer have been either alkylating agents or other compounds that react with DNA nucleophilic sites (eg, cisplatin). HMM, a compound which has structural similarities to classical alkylating agents (3), and which is progressively demethylated in man to yield electrophilic species (8,9), would therefore appear to be an excellent candidate for activity in ovarian cancer. Indeed, single-agent trials demonstrated objective response rates of > 30% for HMM in patients with advanced ovarian cancer.

However, these observations of single-agent responses were made in the early to mid-1970s; since then, two problems with the use of HMM in the treatment of ovarian cancer have become clear.

1. Patients treated with single-agent HMM have *not* been documented by surgical restaging to obtain either a high pathological complete response rate or a significant increase in median survival (13–21). In many of the randomized HMM-containing combination trials there is a paucity of long-term complete responders, and median survival times are similar to the non-HMM-containing combinations.

Since there is some overlap between the toxic effects of HMM and both the alkylators (nausea, vomiting, malaise) and cisplatin (nausea, vomiting, malaise, neuropathy), one must consider the possibility that dose reduction of HMM contained in these combinations necessitated by its incorporation may have seriously compromised the efficacy of this drug.

2. No adequate and completed study to date has demonstrated a role for HMM (ie, determine its contribution to the responses and survival in a comparative fashion) in the treatment of ovarian cancer. There were two randomized studies that isolate HMM in an "addition" manner. The Netherlands study compared a doxorubicin-cisplatin combination with and without HMM (45); the GOG compared melphalan with and without HMM (47,48) (table 5). The Netherlands trial had very few patients, an unusually low complete response rate, and poor median survival figures in both arms. The GOG trial was the best study to date in attempting to isolate a role for HMM in ovarian cancer. In the two HMM-containing arms, 161 patients were evaluable for survival. At the time of the last report, however, there was no significant difference between the arms in either complete response rate or median survival. Based on the data reported, 90% confidence limits for the difference in complete response rates ranged from about 20% in favor of the HMM plus melphalan combination to 4% in favor of melphalan alone. The survival results in the GOG study were based

TABLE 4.—Trials of HMM + cisplatin-containing combinations in patients with advanced ovarian carcinoma who had not received chemotherapy*

Group or institution (ref No.)	Regimen	Interval	No. of evaluable patients	No. of responses			Median survival (mos)	80% confidence interval for CR + PR (%)
				CR	Total	CR + PR (%)		
Portugal (58)	R: CTX 40 mg/kg Day 1	Every 21 days	27	12	18	67	12	53-79
	R: HMM 150-200 mg/m^2 Days 2-10 Cisplatin 40 mg/m^2 Day 1 DOX 40 mg/m^2 Day 1	Every 28 days	26	5	10	39	11	25-53
Mt. Sinai (59,60)	R: HMM 150 mg/m^2 Days 2-7 DOX 30 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 CTX 150 mg/m^2 Days 2-7 (oral)	Every 28 days	37	13	19	51	25	40-63
	R: DOX 50 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 21 days	20	6	11	55	18	39-71
Eastern Cooperative Oncology Group (61)	Melphalan 7 mg/m^2 daily × 5	Every 28 days	153 Total	—	—	—	NR	
	At time of progression					44		
	HMM 150 mg/m^2 Days 8-21 DOX 30 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 21 days	—	—	—	—	NR	
	R: HMM 150 mg/m^2 Days 8-21 CTX 600 mg/m^2 Day 1 DOX 25 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1	Every 28 days	—			63	NR	
Eastern Cooperative Oncology Group (37)	HMM 150 mg/m^2 Days 8-21 DOX 25 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 CTX 600 mg/m^2 Day 1	Every 28 days	21	10	19	90	> 12	77-97
Greece (62)	HMM 6 mg/kg Days 3-16 DOX 30 mg/m^2 Day 1 Cisplatin 50 mg/m^2 Day 1 CTX 300 mg/m^2 Day 1	Every 28 days	38	12	31	81	NR	71-89
Vanderbilt University (41,63)	HMM 150 mg/m^2 Days 1-14 CTX 350 mg/m^2 Days 1, 8 DOX 20 mg/m^2 Days 1, 8 Cisplatin 60 mg/m^2 Day 1	Every 28 days	46	35	44	96	> 19	89-99
Johns Hopkins (64)	HMM 150 mg/m^2 Days 1-14 CTX 500 mg/m^2 Day 1 DOX 4 mg/m^2 Day 1 or Cisplatin 50 mg/m^2 Day 1 on alternating cycles	Every 28 days	29	6	16	55	NR	42-68
Cornell Medical Center (65)	HMM 200 mg/m^2 Days 7-21 DOX 30 mg/m^2 Day 1 Cisplatin 60 mg/m^2 Day 1	Every 28 days	25	12	14	56	18	41-70
	Alternating with							
	HMM 150 mg/m^2 Days 1-14 CTX 150 mg/m^2 Days 1-14 (oral) MTX 40 mg/m^2 Days 1, 8 5-FU 400 mg/m^2 Days 1, 8	Every 28 days						

*Abbreviations as in footnotes to tables 2 and 3.

on 154 patients randomized to receive the combination and 95 randomized to receive melphalan alone. The sample sizes were unbalanced because the melphalan arm was terminated early. The trial was sufficiently large to have been able to detect improvements of about 4–5 months or longer in median survival. The results thus demonstrate that this level of survival benefit from the addition of HMM to melphalan did not exist.

Three groups attempted a randomized trial in which the key variable was the substitution of HMM between arms (44,52,53). The Central Pennsylvania Study Group trial (44), which substituted HMM for cyclophos-

TABLE 5.—Studies that isolate HMM*

Trial		No. of patients	No. of responses		Median survival (mos)
Institution (ref No.)	Design		CR (%)	Total (%)	
	Studies that isolate HMM				
Netherlands (45)	HMM, DOX, Cisplatin	23	1 (4)	4 (17)	5.3
	DOX, Cisplatin	22	1 (5)	5 (23)	6.9
Gynecologic Oncology Group (47,48)	HMM, Melphalan	97	27 (28)	50 (52)	13.5
	Melphalan	64	13 (20)	24 (38)	12.3
	Studies that "almost" isolate HMM				
Central Pennsylvania Study Group (44)	HMM, DOX, Cisplatin	27	6 (22)	11 (41)	7.5
	CTX, DOX, Cisplatin	23	3 (13)	7 (30)	13.5
Milan (52)	HMM, DOX, CTX	49	14 (29)	31 (63)	23
	Cisplatin, DOX, CTX	54	18 (33)	36 (67)	24
Yale (53)	HMM, CTX	20	6 (30)	10 (50)	13.7
	DOX, CTX	17	6 (35)	10 (59)	16.7

*Abbreviations as in footnote to table 2.

phamide in a doxorubicin-cisplatin combination, had low patient numbers, as did the Yale study (53), which substituted HMM for doxorubicin when combined with cyclophosphamide. The Milan trial study design involved a substitution of HMM or cisplatin in a doxorubicin-cyclophosphamide-based combination (52), ie, HMM-doxorubicin-cyclophosphamide (HAC) versus cisplatin-doxorubicin-cyclophosphamide (PAC). The objective response rates were 63% for HAC and 67% for PAC, with median survival of 23 and 24 months, respectively. At a median follow-up time of 36 months, no differences were apparent (52).

There were at least six trials that compared HMM-containing combinations against either melphalan or cyclophosphamide (table 6). The results of these trials were difficult to interpret. The NCI Medicine Branch (50) and the Princess Margaret (54) trials reported more complete responses for the combination treatment arm (NCI Medicine Branch trial also showed a longer median survival for the combination treatment). However, the two Portuguese studies (51,58) reported more complete responses in the single-agent alkylator therapy arm and no difference in median survival over the combinations containing three and four drugs. Hence, these small randomized studies do not convincingly demonstrate that combination therapy is superior to single-agent alkylating therapy in front-line use. They also raise questions about the relative activities of cyclophosphamide and melphalan, although none are large enough to be conclusive. *None of them* demonstrate a contribution of HMM to the activity of the respective combination.

The results from the Eastern Cooperative Oncology Group study are difficult to interpret because patients who failed melphalan as a single agent were treated with an HMM-containing three-drug combination (61). Although the results were only published in abstract form, objective response rates reported for the patients initially treated with the combination were higher than for the group that received single-agent melphalan followed by combination therapy. However, more results are needed before this study can be assessed (61).

Finally, there have been at least seven trials that compared HMM-containing combinations to non-HMM-containing combinations (table 7). Two of these trials have accrued a total number of > 150 patients on the two arms (48,55), but neither has identified a difference in median survival times.

The optimal clinical trial design to determine the activity contribution by a specific drug to a cytotoxic

TABLE 6.—HMM combinations vs single agents*

Trial		No. of patients	No. of responses		Median survival (mos)
Institution (ref No.)	Design		CR (%)	Total (%)	
Georgetown (49)	HMM, CTX, 5-FU	13	7 (54)	11 (85)	NR
	Melphalan	14	3 (21)	8 (57)	NR
NCI Medicine Branch (50)	HMM, CTX, MTX, 5-FU	40	13 (33)	30 (75)	29.0
	Melphalan	37	6 (16)	20 (54)	17.0
Portugal (51)	HMM, CTX, MTX, 5-FU	28	4 (14)	10 (36)	10.0
	CTX	29	7 (24)	18 (62)	11.0
Portugal (58)	HMM, Cisplatin, DOX	26	5 (19)	10 (38)	11.0
	CTX	27	12 (44)	18 (67)	12.0
Princess Margaret (54)	HMM, CTX, MTX, 5-FU	37	7 (19)	NR (NR)	NR
	Melphalan	38	5 (13)	NR (NR)	NR
Eastern Cooperative Oncology Group (61)	HMM, CTX, DOX, Cisplatin	153 Total	(41)	(63)	NR
	Melphalan		NR (NR)	NR (NR)	NR

*Abbreviations as in footnotes to tables 2 and 3.

chemotherapy combination should be tailored around the mechanism(s) of action and toxic effects of the drug. For example, because HMM is relatively nonmyelosuppressive (the dose-limiting toxicity of the majority of the drugs active against ovarian cancer, except cisplatin), an additive rather than a substitution trial would be preferred. On the other hand, cisplatin and HMM have significant overlapping toxic effects to two organ systems, ie, neurological and gastrointestinal, which compromise the ability to perform additive trials without compromising the dose of one or both agents. Although substitution trials could avoid the specific problem of overlapping toxicity between cisplatin and HMM, they would not clarify what HMM added to the activity of the combination unless the study contained a minimum of three arms: "A" plus HMM versus "A" plus cisplatin versus "A," where "A" equals a single agent or combination. Whichever design is selected, however, it is obvious that the number of patients required for reliable results is much greater than the sample sizes used in the large majority of the trials summarized here.

It is disappointing that in spite of a multiplicity of trials that have examined HMM-containing combinations in either front-line therapy or treatment of refractory disease, only one was of adequate size and attempted to isolate the contribution of HMM to combination therapy (47,48). Most of the studies using chemotherapy in advanced ovarian cancer have focused on the following questions.

(*a*) Were drug combinations more effective than single-agent therapy?

(*b*) Was there a difference between response rates of the various alkylating agents?

(*c*) Were cisplatin-containing combinations superior to non-cisplatin-containing combinations?

(*d*) Did any combination have a greater effect upon survival than single-agent cisplatin therapy alone?

Although these issues were extremely important for developing advances in the treatment of this disease, these approaches have neglected to determine whether specific drugs add more to a combination than toxic effects. The latter might necessitate lowering the doses of drugs with a narrow therapeutic index and, in effect, render the combination suboptimal for antineoplastic

TABLE 7.—HMM-containing combinations vs non-HMM-containing combinations*

Trial		No. of patients	No. of responses		Median survival (mos)
Institution (ref No.)	Design		CR (%)	Total (%)	
Central Pennsylvania Study Group (44)	HMM DOX Cisplatin	27	6 (22)	11 (41)	7.5
	R				
	CTX DOX Cisplatin	23	3 (13)	7 (30)	13.5
Gynecologic Oncology Group (47,48)	HMM Melphalan	97	27 (28)	50 (52)	13.5
	R				
	DOX CTX	72	23 (32)	45 (63)	14.2
Milan (52)	HMM DOX CTX	49	14 (29)	31 (63)	23
	R				
	Cisplatin DOX CTX	54	18 (33)	36 (67)	24
M. D. Anderson (55)	HMM DOX CTX	71	20 (28)	22 (31)	26.4
	R				
	Melphalan Cisplatin	82	27 (33)	31 (39)	29.6
Yale (53)	HMM CTX	20	6 (30)	10 (50)	13.7
	R				
	DOX CTX	17	6 (35)	10 (59)	16.7
Princess Margaret (54)	HMM CTX MTX 5-FU	37	7 (19)	NR (NR)	NR
	R				
	CTX DOX Cisplatin	40	12 (30)	NR (NR)	NR
Mt. Sinai (59,60)	HMM DOX Cisplatin CTX	37	13 (35)	19 (51)	25.0
	R				
	DOX Cisplatin	20	6 (30)	11 (55)	18.0

*Abbreviations as in footnotes to tables 2 and 3.

activity. In addition, failure to evaluate the contribution of a drug also hinders the overall building of effective combination regimens.

The published randomized trials did not seek to definitively compare HMM against cisplatin or the alkylators as single agents. The lack of pathologically documented complete responses or published reports of patients who were long-term clinically disease-free survivors following treatment with single-agent HMM is of greater concern.

The role of HMM in the treatment of advanced ovarian cancer remains unclear after > 15 years of study, in spite of the fact that HMM clearly showed single-agent activity in patients with this disease. The inability to either isolate a definitive role for the compound or demonstrate a meaningful effect upon patient survival after 15 years of clinical testing may limit the future use of HMM in the treatment of patients with ovarian cancer as new active compounds are detected in the clinic.

The results of HMM trials in ovarian cancer are used for illustration. However, it should be pointed out that the problems of drug development presented in this manuscript are not unique to HMM.

REFERENCES

1. KAISER DW, THURSTON JT, DUDLEY JR, ET AL. Cyanuric chloride derivatives. II. Substituted melamines. J Am Chem Soc 73:2984-2986, 1951.
2. BRINDLEY C, GESCHER A, LANGDON SP, ET AL. Studies of the mode of action of antitumor triazenes and triazines—III. Metabolism studies on hexamethylmelamine. Biochem Pharmacol 31:625-631, 1982.
3. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine: an evaluation of its role in the therapy of cancer. Cancer 38:27-35, 1976.
4. Unpublished data from the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.
5. BATEMAN AE, PECKHAM MJ, and STEEL GG. Assays of drug sensitivity for cells from human tumors: in vitro and in vivo tests on a xenografted tumour. Br J Cancer 40:81-88, 1979.
6. NOWAK K, PECKHAM MJ, and STEEL GG. Variation in response of xenografts or colo-rectal carcinoma to chemotherapy. Br J Cancer 37:576-584, 1978.
7. BRYAN GT, WORZALLA JF, GORSHE AL, ET AL. Plasma levels and urinary excretion of hexamethylmelamine following oral administration to human subjects with cancer. Clin Pharmacol Ther 9:777-782, 1968.
8. WORZALLA JF, RAMIREZ G, and BRYAN GT. *N*-Demethylation of the antineoplastic agent hexamethylmelamine by rats and man. Cancer Res 33:2810-2815, 1973.
9. WORZALLA JF, KAIMAN BD, JOHNSON BM, ET AL. Metabolism of hexamethylmelamine-ring ^{14}C in rats and man. Cancer Res 34:2669-2674, 1974.
10. D'INCALCI M, BOLIS G, MANGIONI C, ET AL. Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62:2117-2119, 1978.
11. WILSON WL, and DE LA GARZA JG. Phase I study of hexamethylmelamine (NSC-13875). Cancer Chemother Rep 48:49-52, 1965.
12. LOUIS J, LOUIS NB, LINMAN JW, ET AL. The clinical pharmacology of hexamethylmelamine: phase I study. Clin Pharmacol Ther 8:55-64, 1967.
13. WHARTON JT, RUTLEDGE F, SMITH JP, ET AL. Hexamethylmelamine: an evaluation of its role in the treatment of ovarian cancer. Am J Obstet Gynecol 133:833-841, 1979.
14. SMITH JP, and RUTLEDGE FN. Random study of hexamethylmelamine, 5-fluorouracil, and melphalan in treatment of advanced carcinoma of the ovary. Natl Cancer Inst Monogr 42:169-172, 1975.
15. WILSON WL, SCHROEDER JM, BISEL HF, ET AL. Phase II study of hexamethylmelamine (NSC 13875). Cancer 23:132-136, 1969.
16. WILSON WL, BISEL HF, COLE D, ET AL. Prolonged low-dosage administration of hexamethylmelamine (NSC 13875). Cancer 25:568-570, 1970.
17. BONOMI PD, MLADINEO J, MORRIN B, ET AL. Phase II trial of hexamethylmelamine in ovarian carcinoma resistant to alkylating agents. Cancer Treat Rep 63:137-138, 1979.
18. JOHNSON BL, FISHER RI, BENDER RA, ET AL. Hexamethylmelamine in alkylating agent-resistant ovarian carcinoma. Cancer 42:2157-2161, 1978.
19. OMURA GA, GRECO FA, and BIRCH R. Hexamethylmelamine in mustard-resistant ovarian adenocarcinoma. Cancer Treat Rep 65:530-531, 1981.
20. STANHOPE CR, SMITH JP, and RUTLEDGE F. Second trial drugs in ovarian cancer. Gynecol Oncol 5:52-58, 1977.
21. BOLIS G, D'INCALCI M, BELLONI C, ET AL. Hexamethylmelamine in ovarian cancer resistant to cyclophosphamide and adriamycin. Cancer Treat Rep 63:1375-1377, 1979.
22. FISHER RI, and YOUNG RC. Advances in the staging and treatment of ovarian cancer. Cancer 39:967-972, 1977.
23. BAGLEY CM, YOUNG RC, CANELLOS GP, ET AL. Treatment of ovarian carcinomas: possibilities for progress. N Engl J Med 287:856-862, 1972.
24. GRIFFITHS CT. Surgical resection of bulk tumor in the primary treatment of ovarian carcinoma. Symposium on Ovarian Cancer. Natl Cancer Inst Monogr 42:101-104, 1975.
25. OZOLS RF, GARVIN AJ, COSTA J, ET AL. Advanced ovarian cancer: correlation of histologic grade with response to therapy and survival. Cancer 45:572-581, 1980.
26. YOUNG RC, KNAPP RC, and PEREZ CA. Cancer of the ovary. *In* Cancer: Principles and Practice of Oncology (DeVita VT, Hellman S, and Rosenberg SA, eds). Philadelphia, J. B. Lippincott, 1982, p 901.
27. TEMPERO MA, and KESSINGER A. Hexamethylmelamine and melphalan as salvage therapy in advanced ovarian carcinoma. J Surg Oncol 21:159-161, 1982.
28. WHARTON JT, HERSON J, EDWARDS CL, ET AL. Single agent adriamycin followed by combination hexamethylmelamine-cyclophosphamide for advanced ovarian carcinoma. Gynecol Oncol 14: 262-270, 1982.
29. KARDINAL CG, and LUCE JK. Evaluation of hexamethylmelamine and 5-fluorouracil combination in the treatment of advanced ovarian carcinoma. Cancer Treat Rep 61:1691-1693, 1977.
30. SESSAN C, BOLIS G, VALENTE I, ET AL. Hexamethylmelamine, doxorubicin, and cyclophosphamide in advanced ovarian cancer resistant to previous chemotherapy. Cancer Treat Rep 65:172-173, 1981.
31. LOPEZ J, KRIKORIAN J, DIAS S, ET AL. Failure of platinum (DDP)-hexamethylmelamine (HMM) in refractory carcinoma of the ovary. Proc Am Assoc Cancer Res and ASCO 21:431, 1980.
32. VOGL SE, GREENWALD E, KAPLAN BH, ET AL. Ovarian cancer, effective treatment after alkylating-agent failure. JAMA 241: 1908-1911, 1979.
33. TURBOW MM, JONES H, FRIEDMAN MA, ET AL. Chemotherapy of ovarian carcinoma: hexamethylmelamine (HMM) and cisplatinum for patients with persistent disease who fail alkylating-agent and adriamycin therapy. Proc Am Assoc Cancer Res and ASCO 21:361, 1980.
34. VOGL SE, PAGANO M, DAVIS TE, ET AL. Hexamethylmelamine and cisplatin in advanced ovarian cancer after failure of alkylating-agent therapy. Cancer Treat Rep 66:1285-1290, 1982.
35. LUND B, AABO K, RORTH M, ET AL. *cis*-Dichlorodiammineplatinum (II) and hexamethylmelamine in advanced ovarian carcinoma: a phase II study. Eur J Cancer Clin Oncol 18:1089-1092, 1982.
36. ALBERTS DS, HILGERS RD, MOON TE, ET AL. Combination chemotherapy for alkylator-resistant ovarian carcinoma: a preliminary report of a Southwest Oncology Group trial. Cancer Treat Rep 63:301-305, 1979.
37. VOGL SE, BERENZWEIG M, KAPLAN BH, ET AL. The CHAD and HAD regimens in advanced ovarian cancer: combination chemotherapy including cyclophosphamide, hexamethylmelamine, adriamycin and *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 63:311-317, 1979.
38. KANE R, HARVEY H, ANDREWS T, ET AL. Phase II trial of cyclophosphamide, hexamethylmelamine, adriamycin and *cis*-dichlorodiammineplatinum(II) combination chemotherapy in advanced ovarian carcinoma. Cancer Treat Rep 63:307-309, 1979.
39. PIVER MS, LELE S, and BARLOW JJ. Cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin (CHAD) as second-line chemotherapy for ovarian adenocarcinoma. Cancer Treat Rep 65:149-151, 1981.
40. STEPHENS R, COLTMAN C, ROSSOF A, ET AL. *cis*-Dichlorodiammineplatinum(II) in adult patients: Southwest Oncology Group studies. Cancer Treat Rep 63:1609-1610, 1979.
41. GRECO FA, JULIAN CG, RICHARDSON RL, ET AL. Advanced ovarian cancer: brief intensive combination chemotherapy and second-look operation. Obstet Gynecol 58:199-205, 1981.
42. BRUCKNER HW, COHEN CJ, DEPPE G, ET AL. Treatment of chemotherapy-resistant advanced ovarian cancer with a combination of cyclophosphamide, hexamethylmelamine, adriamycin and *cis*-diamminedichloroplatinum (CHAP). Gynecol Oncol 12:150-153, 1981.
43. BRUCKNER HW, COHEN CJ, DEPPE G, ET AL. Ovarian cancer: schedule modification and dosage intensification of cyclophosphamide, hexamethylmelamine, adriamycin, and cis-platin regimen (CHAP-II). Proc ASCO 1:107, 1982.
44. BERNATH A, ANDREWS T, DIXON R, ET AL. Long term follow-up of HAP (hexamethylmelamine, adriamycin, cis-platinum) vs CAP (cyclophosphamide, adriamycin, cis-platinum) in alkylating-agent resistant advanced ovarian carcinoma. Proc ASCO 1:110, 1982.

45. NELJT JP, TEN BOKKEL HUININK WW, VAN DER BURG MEL, ET AL. Combination chemotherapy with or without hexamethylmelamine in alkylating-agent resistant ovarian carcinoma. Cancer 53:1467–1472, 1984.
46. KATZ ME, SCHWARTZ PE, KAPP DS, ET AL. Epithelial carcinoma of the ovary: current strategies. Ann Intern Med 95:98–111, 1981.
47. OMURA GA, BLESSING JA, BUCHSBAUM HJ, ET AL. A randomized trial of melphalan (M) vs melphalan plus hexamethylmelamine (M + H) vs adriamycin plus cyclophosphamide (A + C) in advanced ovarian carcinoma. Proc Am Assoc Cancer Res and ASCO 20:358, 1979.
48. OMURA GA, MORROW CP, BLESSING JA, ET AL. A randomized comparison of melphalan versus melphalan plus hexamethylmelamine versus adriamycin plus cyclophosphamide in ovarian carcinoma. Cancer 51:783–789, 1983.
49. DELGADO G, SMITH FP, MACLAUGHLIN EK, ET AL. Single agent versus combination chemotherapy for ovarian cancer. Am J Clin Oncol 8:33–37, 1985.
50. YOUNG RC, CHABNER BA, HUBBARD SP, ET AL. Advanced ovarian adenocarcinoma. A prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261–1266, 1978.
51. CARMO-PEREIRA J, OLIVEIRA COSTA F, HENRIQUES E, ET AL. Advanced ovarian carcinoma: a prospective and randomized clinical trial of cyclophosphamide versus combination cytotoxic chemotherapy (Hexa-CAF). Cancer 48:1947–1951, 1981.
52. SESSA C, BOLIS G, COLOMBO N, ET AL. Hexamethylmelamine, adriamycin, and cyclophosphamide (HAC) versus *cis*-dichlorodiammineplatinum, adriamycin, and cyclophosphamide (PAC) in advanced ovarian cancer: a randomized clinical trial. Cancer Chemother Pharmacol 14:222–228, 1985.
53. SCHWARTZ PE, LAWRENCE R, and KATZ M. Combination chemotherapy for advanced ovarian cancer: a prospective randomized trial comparing hexamethylmelamine and cyclophosphamide to doxorubicin and cyclophosphamide. Cancer Treat Rep 65:137–141, 1981.
54. STURGEON JFG, FINE S, GOSPODAROWICZ MK, ET AL. A randomized trial of melphalan alone versus combination chemotherapy in advanced ovarian cancer. Proc ASCO 1:108, 1982.
55. EDWARDS CL, HERSON J, GERSHENSON DM, ET AL. A prospective randomized clinical trial of melphalan and cis-platinum versus hexamethylmelamine, adriamycin and cyclophosphamide in advanced ovarian cancer. Gynecol Oncol 15:261–277, 1983.
56. NELJT JP, VAN LINDERT ACM, VENDRIK CPJ, ET AL. Treatment of advanced ovarian carcinoma with a combination of hexamethylmelamine, cyclophosphamide, methotrexate, and 5-fluorouracil (Hexa-CAF) in patients with and without previous treatment. Cancer Treat Rep 64:323–326, 1980.
57. DE PALO G, DEMICHELI R, VALAGUSSA P, ET AL. Prospective study with Hexa-CAF combination in ovarian carcinoma. Cancer Chemother Pharmacol 5:157–161, 1981.
58. CARMO-PEREIRA J, COSTA FO, and HENRIQUES E. Cisplatinum, adriamycin, and hexamethylmelamine versus cyclophosphamide in advanced ovarian carcinoma. Cancer Chemother Pharmacol 10:100–103, 1983.
59. BRUCKNER HW, COHEN CJ, GOLDBERG JD, ET AL. Cisplatin regimens and improved prognosis of patients with poorly differentiated ovarian cancer. Am J Obstet Gynecol 145:653–658, 1983.
60. COHEN CJ, GOLDBERG JD, HOLLAND JF, ET AL. Improved therapy with cisplatin regimens for patients with ovarian carcinoma (FIGO stages III and IV) as measured by surgical end-staging (second-look operation). Am J Obstet Gynecol 145:955–967, 1983.
61. VOGL SE, KAPLAN B, and PAGANO M. Diamminedichloroplatinum (D)-based combination chemotherapy (CT) is superior to melphalan (M) for advanced ovarian cancer (OvCa) when age is $\geq$ 50 and tumor diameter $>$ 2 cm. Proc ASCO 1:119, 1982.
62. RAZIS DV, POULAKOU E, PETOUNIS A, ET AL. Treatment of ovarian cancer with a combination of cis-platinum, adriamycin, cyclophosphamide and hexamethylmelamine. Oncology 39:205–208, 1982.
63. GRECO FA, HONDE KR, JONES HW, ET AL. Advanced ovarian cancer: long-term follow-up after brief intensive chemotherapy. Proc ASCO 3:166, 1984.
64. HERNANDEZ E, ROSENSHEIN UB, VILLAR T, ET AL. Alternating multiagent chemotherapy for advanced ovarian carcinoma. J Surg Oncol 22:87–91, 1983.
65. PASMANTIER MW, COLEMAN M, SILVER RT, ET AL. Six-drug chemotherapy (hexamethylmelamine, doxorubicin, cisplatin, cyclophosphamide, methotrexate, and 5-FU; CHAMP-5) for ovarian carcinoma: alternating sequences of combination regimens. Cancer Treat Rep 69:689–693, 1985.

Radiation in Combination With Intra-arterial Infusion Therapy With Dacarbazine for Metastatic Malignant Melanoma Localized to a Lower Extremity[1]

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Fourteen patients with metastatic malignant melanoma localized to a lower extremity were treated with combined radiotherapy and intra-arterial infusion therapy with dacarbazine. All lesions that were included in radiation fields regressed completely. Two patients relapsed within radiation fields and six relapsed elsewhere. One patient had a serious intimal lesion caused by the catheter. [Cancer Treat Rep 70:1015-1017, 1986]

Metastatic malignant melanoma is ordinarily a therapy-resistant disease. Response rates to radiotherapy vary with tumor size, localization, and fractionation. Local control with radiotherapy alone is usually not achieved. Chemotherapy has been reported to induce remissions in approximately 20% of patients; dacarbazine (DTIC) is slightly more effective than other drugs (1). The response rate for DTIC at The Norwegian Radium Hospital is 14% (unpublished data).

In experimental studies using human malignant melanomas passed serially in athymic nude mice, combined ^{60}Co radiation and DTIC were more effective than either treatment alone (2).

Intra-arterial infusion of chemotherapeutic agents delivers a high concentration of the particular agent to the perfused area. Einhorn et al (3) have reported an overall response rate of 41% in isolated inoperable melanoma lesions.

In some cases of metastatic malignant melanoma, metastases, usually cutaneous or subcutaneous, remain localized to one extremity for longer periods of time (often years) before distant metastases develop. Usually both the primary and metastases are localized to the lower extremity. Such patients have earlier been treated with surgical extirpation of metastases, in many cases combined with limited radiation fields. However, in most instances, the ultimate outcome has been amputation of the extremity and/or widespread dissemination.

Since 1977, 14 patients have been selected for treatment with a combination of radiotherapy and intra-arterial DTIC. All achieved a complete remission, in one patient lasting $>$ 108 months. In one case, intra-arterial catheterization was complicated by serious arterial damage.

MATERIALS AND METHODS

The main patient characteristics are summarized in table 1. All 14 patients had metastatic melanoma localized to one lower extremity. Eleven of the patients were women. The median age was 58 years. The median disease-free interval (from removal of primary until metastases) was 35 months and the median time from first metastases until treatment start was 27 months. In all cases the primary melanoma had been localized to the same extremity and excised. The patients were without evidence of distant metastases. Catheterization was performed in the brachial artery in 13 patients and through the groin in one patient. The tip of the catheter was localized in either the femoral or the iliac artery of the metastatic extremity. Eleven patients received 150 mg of DTIC immediately before and the same dose 2 hours after radiotherapy for the first 5 days. In two patients, the same dose schedule was delivered for 14 days. For the patient who had catheterization through the groin, 400 mg of DTIC was given 2 hours after radiotherapy every other day for four times. DTIC was dissolved in 0.9% saline in a volume of 1 L delivered as a 30-minute infusion. The catheter was kept open by continuous heparinized saline infusion, using an inflatable cuff (Fenwal bag) to keep the pressure above arterial (approximately 300 mm Hg). Irradiation was delivered by either a ^{60}Co unit or a linear accelerator with bolus covering skin lesions. The dose of radiation varied somewhat (table 1). In all but two patients (Nos. 8 and 9), the whole leg, or if necessary, the whole extremity, was irradiated. For these two patients, several smaller fields were given. Following the initial combined treatment, 13 patients received 800 mg/m^2 of DTIC intra-arterially one to eight times as an infusion

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TABLE 1.—Patient characteristics

Patient No.	Sex	Age (yrs)	Disease-free interval (mos)	Mos from 1st metastasis to treatment start	Metastases from 2 nodes	No. of metastases	Radiation dose	W
1	F	51	12	60	−	> 5	3 Gy × 12	
2	F	62	1	3	+	> 5	4 Gy × 10	
3	F	69	7	28	−	< 5	4 Gy × 8	
4	F	51	156	33	+	< 5	3 Gy × 12	
5	F	55	34	60	−	> 5	3 Gy × 12	
6	F	69	36	10	−	< 5	3 Gy × 12	
7	F	62	36	60	+	> 5	4,8 Gy × 7	
8	F	51	12	60	−	> 5	4,8 Gy × 7	
9	M	70	1	9	+	> 5	4,8 Gy × 7	
10	M	55	Unknown	10	−	> 5	36 Gy × 12	
11	F	62	54	15	−	> 5	3 Gy × 10	
12	F	62	62	18	−	> 5	3 Gy × 12	
13	F	52	6	26	−	> 5	3 Gy × 12	
14	M	56	108	36	+	< 5	3 Gy × 12	

*CR = complete remission.
†Mos after therapy and additional therapy are shown in parentheses.
‡Still in remission.

cteristics, treatment, and results*

tion e	Wks until response	Response	Duration of CR within radiation field (mos)	Relapses†	
				Within radiation field	Outside radiation field
× 12	24	CR‡	108‡	–	–
× 10	20	CR‡	24‡	–	–
× 8	4	CR‡	27‡	–	–
× 12	5	CR‡	31‡	–	–
× 12	22	CR‡	37‡	–	–
× 12	5	CR‡	25‡	–	–
× 7	24	CR‡	24‡	–	–
× 7	24	CR‡	96‡	–	Skin (24, surgery)
× 7	24	CR‡	15‡	–	Skin (12, hyperthermic perfusion)
× 12	28	CR	15‡	–	Abdominal nodes, lung (15, interferon)
× 10	12	CR	24‡	–	Brain (radiation)
× 12	28	CR	14‡	–	Regional nodes (4, surgery)
× 12	24	CR	7	+ (7, no therapy)	
× 12	6	CR	15	+ (15, surgery)	Abdominal nodes (10, carboplatin)

over 1 hour every 4 weeks. One patient refused further treatment after the combined treatment had been given due to religious conviction. Complete response was defined as disappearance of all visible or palpable disease for at least 1 month.

RESULTS

All of the 14 patients achieved complete remission; nine of these patients are still in remission (table 1). Two patients (Nos. 8 and 9) who received several smaller fields relapsed between the fields after 51 and 13 months. Two patients relapsed inside the radiated area. Following additional treatment, none of the patients except two have evidence of disease locally or elsewhere (table 1).

In connection with removal of the catheter from the brachial artery after the last DTIC injection concurrent to radiotherapy, one patient (No. 13) developed a pulseless arm. The catheter was then inserted through the groin, resulting in serious impairment of the circulation to both extremities. An attempt to restore circulation operatively failed. She then developed paraparesis, probably due to ischemic injury to the medulla, and her disease relapsed locally within the radiation field 7 months after the start of treatment.

For doses of DTIC < 800 mg/m^2, toxicity was moderate. For doses of 800 mg/m^2, nausea and vomiting of grade 2 (World Health Organization criteria) was regularly seen. Nadir blood counts were not measured. No courses were postponed due to myelosuppression.

DISCUSSION

The patients treated in this study are highly selected. They all had melanoma metastases localized to one lower extremity with no evidence of distant metastases. The metastases remained to one extremity for a relatively long time, reflected by a median time of 27 months from first metastases until the start of treatment. A serious complication related to catheterization occurred in one patient. Such complications related to arterial catheterization are generally very rare. The possibility that chemotherapy may have contributed has to be considered. The patient was a heavy smoker; therefore, predisposing factors cannot be ruled out. The two patients who received several smaller radiation fields to the extremity both relapsed between the fields. Therefore, radiotherapy probably should be given to the whole leg, thigh, or if necessary, the whole extremity.

The relative contributions of the radiotherapy and chemotherapy are unclear and are not solved by this study. It is also unclear how many courses of chemotherapy should be given in addition to the initial combined treatment. Likewise, optimal fractionation and accumulated dose of radiotherapy are unclear. These questions can only be solved by multicenter randomized studies. The current treatment that has been chosen for further evaluation is shown in table 2.

TABLE 2.—Current treatment

Treatment	Dose
Induction	
Radiation therapy	3 Gy × 12
Chemotherapy	150 mg/m^2 of DTIC intra-arterially divided in 2 fractions, before and 2 hrs after radiation on Days 1–5
Consolidation	
Chemotherapy	800 mg/m^2 of DTIC intra-arterially every 4 weeks × 3

With regional hyperthermic perfusion with DTIC (4) or melphalan (5,6), remission rates of 50%–70% have been reported, with a complete remission rate of approximately 30%. These patients seem to have been selected on similar premises as those in the current study. We therefore find it pertinent to point out that despite several unresolved questions, combined radiotherapy and intra-arterial DTIC given to patients with metastatic melanoma localized to a lower extremity are highly effective, with a complete remission rate and duration well above those reported with hyperthermic perfusion.

REFERENCES

1. COMUS RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
2. ROFSTAD EK, BRUSTAD T, JOHANNESSEN JV, ET AL. Effect of cobalt-60 gamma rays and DTIC (5-(3,3 dimethyl-1-triazeno)-imidazole-4-carboxamide) on human malignant melanomas grown in athymic nude mice. Br J Radiol 50:314–320, 1977.
3. EINHORN LH, MCBRIDE CM, LUCE JK, ET AL. Intraarterial infusion therapy with 5-(3,3 dimethyl-1-triazeno)-imidazole-4-carboxamide (NSC 45388) for malignant melanoma. Cancer 32:749–755, 1973.
4. VAGLINI M, BELLI F, MAROLDA R, ET AL. Hyperthermic perfusions with DTIC in melanoma patients. *In* the First International Conference on Skin Melanoma, Venice, May 6–9, 1985, p 85.
5. ROSIN RD, and WESTBURY G. Twenty-four years experience using isolated limb perfusion for recurrent malignant melanoma. *In* the First International Conference on Skin Melanoma, Venice, May 6–9, 1985, p 86.
6. GÖHL J, TONALE J, and SCHÜXK R. Indications and results of regional hyperthermic perfusion in malignant melanoma. *In* the First International Conference on Skin Melanoma, Venice, May 6–9, 1985, p 88.

BRIEF REPORTS

Ineffectiveness of Tamoxifen in Advanced Endometrial Carcinoma After Failure of Progestin Treatment[1,2]

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Between May 1980 and November 1983, 46 eligible patients with advanced, objectively evaluable endometrial carcinoma were treated with tamoxifen, 10 mg twice daily. Twenty-two of the patients had previously received progestational agents. None of the progestin-refractory patients experienced tumor regression on tamoxifen treatment, while five of the 24 previously untreated with progestins did experience tamoxifen-induced tumor regression. Of the five patients previously treated with cytotoxic drugs, only one experienced objective tumor regression on tamoxifen. Although 28 of these 46 patients were completely ambulatory at the beginning of tamoxifen treatment, only 26% were alive 1 year later; median survival for the entire group was only 120 days. Tamoxifen in this dose cannot be recommended routinely as effective secondary treatment for patients with progestin-refractory advanced endometrial carcinoma. [Cancer Treat Rep 70:1019-1020, 1986]

Following the report in 1961 by Kelley and Baker (1) that synthetic progestational agents could induce objective tumor regression in patients with metastatic endometrial carcinoma, this hormonal approach became widely accepted as the initial systemic therapy for patients with well-differentiated metastatic cancer of endometrial origin. Then in 1975, Jordan and Hoerner (2) reported that tamoxifen could inhibit the binding of estradiol by the estrogen receptor protein in endometrial carcinoma, and in 1979 Swenerton et al (3) reported therapeutic effects of tamoxifen in four of seven progestin-refractory patients with advanced endometrial carcinoma. In order to better evaluate tamoxifen in the therapy of advanced endometrial carcinoma, we performed a multi-institutional phase II study involving patients with progestin-refractory tumors and others who had not received progestin treatment.

MATERIALS AND METHODS

Between May 1980 and November 1983, 50 patients with advanced, objectively evaluable endometrial carcinoma were treated with tamoxifen, 10 mg twice daily, until signs of disease progression appeared. All of the patients had disease not amenable to control by surgical or radiation treatments, and none had other active cancers at the time tamoxifen treatment began. Patients were required to have Eastern Cooperative Oncology Group performance status scores of 3 or better and to have expected survival of at least 2 months. Each of the patients who had previously received progestin treatment had experienced definite disease progression prior to beginning tamoxifen treatment. Estrogen receptor content of tumors was requested for participating patients, but this was not obligatory. All variants of endometrial carcinoma were accepted including adenosquamous tumors. No tumors containing sarcomatous elements were included.

For both measurable and evaluable disease, designation of complete regression required total disappearance of all evidence of endometrial cancer. Partial tumor regression was defined as a reduction of at least 50% in the products of the longest perpendicular diameters of measured lesions, or definite decrease in tumor size agreed upon by two investigators for evaluable lesions. Disease progression was identified by at least a 25% increase in the products of the longest perpendicular diameters of measured lesions or definite increase in tumor size agreed upon by two investigators for evaluable disease, or by the appearance of a new lesion during treatment in either case. Disease stability was iden-

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tified when neither progression nor regression of disease could be declared according to the preceding criteria.

RESULTS

Among the 50 patients enrolled in this study, one was excluded because of an active second primary cancer; two had inadequate documentation of active endometrial cancer; and one was ineligible because no signed consent form could be identified. Thus, 46 patients, with a median age of 68 years (range, 54–88), were available for analysis. Twenty-two of these patients had progestin-refractory tumors and the other 24 had not received progestin treatment. Only five patients had received cytotoxic drug treatments. The principal site of metastatic disease was the pelvic-abdominal area in 25 cases, the lungs in 15, and various other sites in six. Cytoplasmic estrogen receptor data were available on eight patients (five, positive; three, negative). The one patient among these eight who experienced objective tumor regression had estrogen receptor-positive status.

Although stability of disease for at least 2 months was observed in six of the 22 progestin-refractory patients, none of these patients experienced objective tumor regression during tamoxifen treatment. The one patient who had experienced definite objective regression during progestin treatment had immediate disease progression on tamoxifen. Among those patients who had not previously received progestins, 11 had disease stability for at least 2 months and five others experienced objective tumor regression (one, complete). Included among the five who achieved objective tumor regression were two with pelvic-abdominal disease, two with pulmonary disease, and one with principal metastases to other sites. Among the 14 patients with poorly differentiated tumors, only one experienced objective tumor regression, while four of the 32 moderately or well-differentiated tumors regressed. The single objective regression of a poorly differentiated carcinoma occurred in a 60-year-old woman with predominant pelvic-abdominal disease of unknown estrogen receptor content who had previously received neither progestins nor cytotoxic drugs.

Median time to disease progression was 57 days and median survival after beginning tamoxifen treatment was 120 days. Anorexia occurred in seven patients and some nausea developed in three of these plus four others. This progressed to actual vomiting in two. Hot flashes were aggravated in four patients.

DISCUSSION

With an overall 11% objective regression rate (21% among patients not previously treated with progestins), our therapeutic results appear slightly more hopeful than those recently reported by the Gynecologic Oncology Group (GOG) (4). When compared more critically, however, our zero regression rate among the 22 progestin-refractory patients is quite consistent with their failure to observe any objective tumor regressions among 19 patients heavily pretreated with progestins and/or cytotoxic drugs. Neither the GOG results nor ours tend to confirm the preliminary observations by Swenerton (5) and by Bonte et al (6), which suggested that tamoxifen might be an effective treatment for progestin-refractory endometrial carcinoma. These authors reported, respectively, one complete plus two partial regressions among ten such patients (5) and "complete or marked" tumor regression in five of nine progestin-refractory patients (6). Hald et al (7) from Denmark and Kauppila and Vihko (8) from Finland have reported tamoxifen-induced objective tumor regression in one of three and one of one progestin-refractory cases, respectively.

Despite the therapeutic benefit which may occasionally result, tamoxifen, in this conventional dose, cannot be recommended routinely as effective secondary treatment for patients with progestin-refractory advanced endometrial carcinoma.

REFERENCES

1. KELLEY R, and BAKER WH. Progestational agents in the treatment of carcinoma of the endometrium. N Engl J Med 264:216–222, 1961.
2. JORDAN VC, and HOERNER S. Tamoxifen and the human carcinoma 8S estrogen receptor. Eur J Cancer 11:205–206, 1975.
3. SWENERTON KD, SHAW D, WHITE GW, ET AL. Treatment of advanced endometrial carcinoma with tamoxifen. N Engl J Med 301:105, 1979.
4. SLAVIK M, PETTY WM, BLESSING JA, ET AL. Phase II clinical study of tamoxifen in advanced endometrial adenocarcinoma: a Gynecologic Oncology Group study. Cancer Treat Rep 68:809–811, 1984.
5. SWENERTON KD. Treatment of advanced endometrial adenocarcinoma with tamoxifen. Cancer Treat Rep 64:805–811, 1980.
6. BONTE J, IDE P, BILLIET G, ET AL. Tamoxifen as a possible chemotherapeutic agent in endometrial adenocarcinoma. Gynecol Oncol 11:140–161, 1981.
7. HALD I, PAGEL J, SALIMTSCHIK M, ET AL. Tamoxifen treatment of advanced endometrial carcinoma: a phase II study. Rev Endocrine-Related Cancer Suppl 11:43–46, 1982.
8. KAUPPILA A, and VIHKO R. Endometrial carcinoma insensitive to progestin and cytotoxic chemotherapy may respond to tamoxifen. Acta Obstet Gynecol Scand 60:589–590, 1981.

First-Line Mitoxantrone Chemotherapy for Advanced Breast Cancer[1]

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Results of first-line mitoxantrone chemotherapy for advanced breast cancer are presented. Full dose (14 mg/m² iv every 3 weeks) was given to 22 patients who had received no previous adjuvant chemotherapy; five (23%) partial responses were seen. No responses were seen in eight patients who had failed adjuvant chemotherapy, of whom seven received full dose. No responses were seen in nine patients receiving lower doses (mean, 10 mg/m²). The overall response rate was 13%. Treatment was well tolerated. Moderate or severe nausea/vomiting and alopecia were infrequent. One patient developed heart failure without predisposing risk factors. [Cancer Treat Rep 70:1021-1022, 1986]

Present chemotherapy regimens for advanced breast cancer yield variable but often disappointing results. Although response rates with combination chemotherapy are usually > 50%, the majority of these responses are partial. Furthermore, the median durations of response and survival are approximately 9 and 21 months, respectively (1). Mitoxantrone was introduced on an investigational basis in Canada in 1983. We report here the results of treatment of advanced breast cancer with mitoxantrone as first-line chemotherapy using a single-dose schedule of 14 mg/m² iv repeated every 3 weeks. This regimen was utilized in the largest previous phase II study, which yielded a 35% response rate (2).

PATIENTS AND METHODS

Patients with measurable locally recurrent or metastatic breast cancer who ordinarily would have been offered systemic combination chemotherapy were eligible, excluding patients with brain or pulmonary lymphangitic metastases. Eligibility criteria included: wbc count > 4000/mm³, platelet count > 120,000/mm³, no concurrent hormone manipulation, no previous chemotherapy for metastatic disease, no serious cardiovascular disease, serum creatinine < 1.5 mg/100 ml, and baseline ECG and left ventricular ejection fraction (LVEF) measurement by gated nuclear angiography. Mitoxantrone was given at 14 mg/m² iv over 30 minutes every 21 days.

The dose was reduced by 2 mg/m² if the wbc count was 2000-4000/mm³ and/or the platelet count was 70,000-120,000/mm³. If there was no hematologic toxicity after two courses and the disease had not responded, the dose was escalated by 2 mg/m². Responses were defined according to standard criteria (3).

RESULTS

Thirty-eight patients, three premenopausal and 35 postmenopausal, were treated. The median age was 59 years (range, 30-75). Performance status according to Eastern Cooperative Oncology Group criteria was 0, 1, 2, and 3 (mean, 1.2) in four, 24, nine, and one patient, respectively. Hormone therapy had been given to 22 patients. A minimum of 4 weeks elapsed from the termination of hormone therapy or radiotherapy to the beginning of mitoxantrone therapy, except in patients with no response to hormone therapy, for whom a minimum of 1 week elapsed. Radiotherapy after primary surgery had been given to 16 patients. Adjuvant chemotherapy had been given to eight patients: five received cyclophosphamide, methotrexate, and 5-FU (CMF), two received melphalan and 5-FU, and one received doxorubicin and cyclophosphamide. Multiple metastases were present in 28 patients. Lymph node, skin, lung, liver, and bone metastases were present in 13, 10, 10, 17, and 23 patients, respectively.

All patients were evaluated for response and toxicity. Full dose was given to 29 patients with performance status of 0, 1, 2, and 3 in three, 19, six, and one case, respectively. Nine patients received reduced doses because of previous radiation therapy (five), hyper-

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bilirubinemia (three), and age > 70 years (one). The mean and median numbers of courses received were five and two, respectively. Two patients had subsequent doses increased by 2 mg/m^2.

Partial responses (PR) were seen in five patients [three estrogen receptor (ER)-negative, two ER-unknown], lasting 8, 9, 20, 41, and 60 weeks, with survival of 15, 6+, 17, 14+, and 23+ months, respectively. Stable disease was seen in eight patients (six ER-positive, one ER-negative, and one ER-unknown), lasting 12–60+ weeks (mean, 32), with survival of 8, 9, 9, 10, 12+, 15, 15+, and 18+ months. Progressive disease was seen in 25 patients, with survival of 1–15 months (median, 3). All responding patients and seven stable patients received full dose. Responses were seen in chest wall (four patients), skin (one), lung (three), lymph node (two), and bone (three) metastases. All patients failing previous adjuvant chemotherapy had progressive disease. In patients with local failure of adjuvant radiotherapy, three had PR and four had stable disease.

Subsequently, CMF was given to 12 patients; this resulted in one PR and two minor responses. Doxorubicin was given to three patients; one PR was observed in a patient who had had progressive disease while receiving mitoxantrone.

Toxicity

Toxicity in 193 treatment cycles was assessed by standard World Health Organization criteria (4). Leukopenia was frequent (52%) but never severe, and was not associated with serious infection. Ranges of mean and minimum treatment day counts were 2900–7000 and 1500–2900/mm^3 for wbcs and 105,000–288,000 and 101,000–135,000/mm^3 for platelets. Mild to moderate stomatitis and diarrhea occurred in nine and 11 patients, respectively. Nausea/vomiting was absent in 13, mild in 14, moderate in nine, and severe in two patients. Alopecia was absent in 13, mild in 15, moderate in eight, and severe in two patients. LVEF reductions by 50%, 43%, 18%, and 14% occurred in four patients at total mitoxantrone doses of 72, 333, 139, and 108 mg/m^2, respectively. The patient with a 50% reduction in LVEF had no pre-existing heart disease, had normal baseline ECG and LVEF, and developed refractory congestive cardiomyopathy that was confirmed at autopsy.

DISCUSSION

This study confirms activity of mitoxantrone in advanced breast cancer. The response rate of 13% (five of 38 patients), along with previous reports (2,5–8), suggests that its activity is similar to most other agents, with the exception of doxorubicin (9). However, its toxicity appears less than that usually seen with doxorubicin, with less nausea and vomiting, alopecia, stomatitis, and myelosuppression and no reports of cellulitis, vesication, or tissue necrosis after extravasation.

REFERENCES

1. BONADONNA G, and VALAGUSSA P. Chemotherapy of breast cancer: current views and results. Int J Radiat Oncol Biol Phys 9:279–297, 1983.
2. SMYTHE JF, CORNBLEET MA, STUART-HARRIS RC, ET AL. Mitoxantrone as first-line chemotherapy for advanced breast cancer: results of a European collaborative study. Semin Oncol 11(suppl 1):15–18, 1984.
3. HAYWARD JL, RUBENS RD, CARBONE PP, ET AL. Assessment of response to therapy in advanced breast cancer. Br J Cancer 35:292–298, 1977.
4. WORLD HEALTH ORGANIZATION. Handbook for Reporting Results of Cancer Treatment. WHO Offset Publ No. 48. Geneva, WHO, 1979.
5. YAP HY, BLUMENSCHEIN GR, SCHELL FC, ET AL. Dihydroxyanthracenedione: a promising new drug in the treatment of metastatic breast cancer. Ann Intern Med 95:694–697, 1981.
6. NEIDHART JA, GOCHNOUR D, ROACH RW, ET AL. A comparative trial of mitoxantrone and doxorubicin in patients with minimally pretreated breast cancer. Semin Oncol 11(suppl 1):11–14, 1984.
7. LENZHOFER R, RAINER H, GRANINGER W, ET AL. Mitoxantrone as initial chemotherapy in advanced breast cancer: preliminary report. *In* Proceedings of the 13th International Chemotherapy Congress, Vienna, 1983, pp 41–44.
8. LEONARD RCF, FARQUHAR DL, KAYE SB, ET AL. Comparison of mitoxantrone with Adriamycin in combination chemotherapy for advanced breast cancer. *In* Proceedings of the 3rd European Conference on Clinical Oncology, Stockholm, 1985, Abstr 250.
9. HOOGSTRATEN B, and FABIAN C. A reappraisal of single drugs in advanced breast cancer. Cancer Clin Trials 2:101–109, 1979.

Phase II Trial of Retinol in Patients With Advanced Cancer[1,2]

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For the Puget Sound Oncology Consortium

We have completed a phase II trial of oral retinol (200,000 units/m^2) in 65 patients with advanced cancer. All patients were followed at 4-week intervals for tumor response and clinical or chemical evidence of toxicity. Seventeen patients had non-small cell lung cancer, 15 had melanoma, 12 had adenocarcinoma of the colon, and 17 had miscellaneous tumors. There were one partial response, five mixed responses, two patients with stable disease, and 47 patients with progressive disease. Thirty-seven of 60 patients reported no side effects; 13 developed mild cutaneous symptoms; and 12 developed reversible central nervous system symptomatology. Oral retinol appears to have limited activity in patients with advanced cancer. [Cancer Treat Rep 70:1023-1024, 1986]

The retinoids (vitamin A and its family of synthetic and naturally occurring compounds) are active in decreasing the number and size of carcinogen-induced animal tumors and can also inhibit tumor growth and induce differentiation (1-3). In humans, retinol and 13-*cis* retinoic acid have shown clinical activity in squamous cell cancers, melanoma, and mycosis fungoides (4-6). Because of the relatively low toxicity potential of these compounds and the suggestion of clinical activity, we have completed a phase II trial of retinol in patients with advanced cancer.

MATERIALS AND METHODS

Sixty-five patients with a histologic diagnosis of cancer who were refractory to standard therapy, had measurable disease, and had a life expectancy of > 8 weeks were given oral retinol in a daily dose of 200,000 units/m^2 (Aquasol A; Armour Pharmaceutical, Kankakee, IL). (Recommended daily allowance for retinol is 4000-5000 units.) Patients with brain metastasis, oral retinol supplementation of > 10,000 units/day, or serum bilirubin > 2.0 mg/dl were not eligible. All patients were evaluated at 4-week intervals for toxicity and tumor response (Southwest Oncology Group criteria). Radionuclide liver/spleen scan or computerized tomographic scans were obtained before treatment and repeated every 4 months. Patients were maintained on retinol for at least 8 weeks unless they developed progressive disease or severe toxic effects. Patients' characteristics are outlined in table 1.

RESULTS AND DISCUSSION

Fifty-five of the 65 patients were evaluable for response. There were no complete responses, one partial response, five mixed responses, two patients with stable disease, and 47 patients with progressive disease. Although there was insufficient accrual within each tumor type to establish response rates, there was adequate accrual for non-small cell lung cancer, adenocarcinoma of the colon, and melanoma to rule out a major response rate of > 20%.

A 38-year-old female with melanoma and multiple 0.1-2.0-cm skin metastases had a partial response characterized by a > 50% decrease in diameter of all skin metastases lasting 19 months. Five additional patients with multiple primary skin cancers (basal cell/squamous cell) had mixed responses, with some lesions decreasing in size and others remaining unchanged, suggesting that this compound may have some biologic activity in low-grade epithelial skin cancers. Two patients had stable disease. One patient with progressive squamous cell lung cancer had disease stabilization for 13 months, which subsequently progressed when retinol was stopped. An additional melanoma patient with multiple skin metastases had disease stabilization for 1 year.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	65
Males/females	34/31
Mean age in yrs (range): 69 (33–92)	
Mean performance status (range): 70 (30–90)	
Previous treatment	
None	15
Chemotherapy	37
Radiation therapy	29
Tumor type	
Lung	
Non-small cell	15
Mesothelioma	2
Melanoma	15
Colon	
Adenocarcinoma	12
Skin	
Squamous cell	3
Basal cell	2
Soft tissue sarcoma	5
Head and neck	
Squamous cell	4
Gynecologic	
Ovarian; endometrial	4
Genitourinary	
Renal; prostate; bladder	3

TABLE 2.—Side effects of oral retinol in 60 patients receiving 200,000 units/m^2/day

Side effect	No. of patients (%)
Skin	13 (22)
Mean onset in days (range): 90 (12–638)	
Symptoms	
Pruritus	6
Dryness, scaling, or chapped lips	7
Patchy depigmentation	2
CNS	12 (20)
Mean onset in days (range): 12 (1–240)	
Symptoms	
Confusion	4
Headaches	3
Depression	3
Dizziness	1
Hallucination	1
Seizures	1
Papilledema	1
Nausea	1
None	37 (62)
Mean duration of therapy in days (range): 55 (5–120)	

Twenty-one patients had adequate serial fasting plasma/serum studies to evaluate lipid and hepatic functions. Triglycerides rose a mean of 114.6 mg/dl from pretreatment values ($P < 0.0005$). There was no change in plasma cholesterol ($P > 0.05$). Both SGOT and alkaline phosphatase increased during treatment; however, in each case the increases were accompanied by progressive hepatic tumor. In patients without hepatic metastasis, there was no change in either SGOT or alkaline phosphate. No changes were seen in other serial blood chemistry, hematologic, coagulation, or urinary studies.

One patient with melanoma developed hepatomegaly on liver/spleen scan after 8 months of retinol. Alkaline phosphatase and SGOT fell during treatment. Because of stable disease, she continued retinol for an additional 4 months without evidence of clinical or chemical toxicity. Although only one patient had evidence of hepatic toxicity during this study, it is likely that others would have developed toxicity with chronic administration of this dose of retinol.

Subjective toxicity was evaluable in 60 patients. Thirty-seven of 60 patients (62%) reported no side effects (table 2). Thirteen patients reported cutaneous symptoms of pruritis and dryness (mean onset, 90 days). Two melanoma patients developed patchy depigmentation of the skin of the arms, face, and upper chest.

Twelve patients developed acute CNS symptoms which included confusion, headaches, and depression (mean onset, 12 days). One patient developed seizures and was found to have brain metastasis. In all cases, toxicity resolved within 2 weeks of stopping retinol. It is unclear why these 12 patients developed acute CNS toxicity. We did not measure the serum concentrations of retinol, retinol binding protein, or the retinyl esters; however, in our previous phase I trial, we could not document a correlation between CNS toxicity and elevated plasma concentrations of retinol or the retinyl esters (6).

CONCLUSION

We conclude that oral retinol has little activity in advanced cancer, although it may have some growth-modulating activity in low-grade skin cancers. Side effects occurred in 38% of the subjects and, although usually mild, 20% developed acute CNS symptoms.

REFERENCES

1. MAYER H, BOLLAG W, HANNI R, ET AL. Retinoids, a new class of compounds with prophylactic and therapeutic activities in oncology and dermatology. Experientia 34:1105, 1978.
2. TROWN PW, BUCK MJ, and HANSEN R. Inhibition of growth and regression of a transplantable rat chondrosarcoma by three retinoids. Cancer Treat Rep 60:1647–1653, 1976.
3. SIDELL N. Retinoic acid induced inhibition and morphological differentiation of human neuroblastoma cells in vitro. JNCI 68:589, 1982.
4. MEYSKENS FL, JR, GILMARTIN E, ALBERTS DS, ET AL. Activity of isotretinoin against squamous cell cancers and preneoplastic lesions. Cancer Treat Rep 66:1315–1319, 1982.
5. KESSLER JF, MEYSKENS FL, JR, LEVINE N, ET AL. Treatment of cutaneous T-cell lymphoma. Lancet 2:629, 1980.
6. GOODMAN GE, ALBERTS DS, EARNEST DL, ET AL. Phase I trial of retinol in cancer patients. J Clin Oncol 1:394, 1983.

Combination Therapy With Mitoxantrone and Etoposide in Refractory Acute Myelogenous Leukemia[1]

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We have investigated the efficacy of mitoxantrone in combination with etoposide in patients with refractory acute myelogenous leukemia. The regimen consisted of 10 mg/m²/day of mitoxantrone given iv on Days 1–5; and 100 mg/m²/day of etoposide as short infusion, initially on Days 1–3 and extended to Days 4 and 5 as appropriate. Of the 26 patients treated with this combination, nine (34.6%) have achieved complete remission and two have achieved partial remission. The median duration of continuous complete remission was 85 days (range, 21–183+). Toxicity was mild and only one case of early death was observed. This combination seems to be an active regimen in refractory acute myelogenous leukemia, and its incorporation in front-line therapy seems warranted. [Cancer Treat Rep 70:1025–1027, 1986]

Despite improvement in the complete remission (CR) rate of adult acute myelogenous leukemia (AML), the majority of patients will relapse within 2 years of attaining CR (1–3). Recently, the effectiveness of mitoxantrone in relapsed and refractory acute leukemia has been reported (4–7). Etoposide has also been shown to be effective in the treatment of AML as a single agent (8,9). This multicenter phase I/II study was undertaken to evaluate the efficacy of the combination of mitoxantrone and etoposide in refractory AML.

MATERIALS AND METHODS

Adult patients (> 15 years of age) with refractory AML were eligible for the study if they had normal liver and renal functions and did not have active infections or signs of cardiac insufficiency. Patients with the following were considered to be refractory: (*a*) failure to achieve CR after two induction courses of conventional TAD, which consists of 6-thioguanine (100 mg/m²/12 hours for 7 days), cytarabine (100 mg/m²/12 hours for 9 days), and daunorubicin (45 mg/m²/day for 3 days); (*b*) relapse within the first 6 months after achieving the first CR; (*c*) failure to achieve a second CR by one other induction course in first relapse, occurring > 6 months after CR; (*d*) first relapse under continuous maintenance therapy; and (*e*) second or subsequent relapses.

Mitoxantrone was given at a dosage of 10 mg/m²/day on Days 1–5 as rapid iv infusion (15 minutes). The initial schedule of etoposide was 100 mg/m²/day on Days 1–3. In subsequent groups of at least five patients each, escalation of etoposide was achieved by increasing the number of days of therapy to a maximum of 5 days. The cardiac ejection fraction was assessed by echocardiography or by multigated cardiac scan. If no CR was achieved with the first course and there was a blast reduction of > 50%, judged when marrow cellularity was ≥ 2+, a second course was to be administered. Patients who achieved CR were to be given one course of consolidation therapy, which consisted of 8 mg/m²/day of mitoxantrone on Days 1–5, 75 mg/m²/day of etoposide on Days 1–5, and 75 mg/m²/12 hours of cytarabine on Days 1–5. The study protocol was reviewed by each institution's Protocol Review Committee and informed consent was obtained before therapy. CR was defined as < 5% blasts in a normocellular bone marrow with a granulocyte count of > 1000/μl and a platelet count of > 100,000/μl in peripheral blood and with normal physical findings. Partial remission (PR) was defined as either < 5% blasts in marrow but incomplete recovery of blood cell counts or 5%–25% blasts in bone marrow but normalization of platelet and granulocyte counts in peripheral blood. Toxicity was assessed by World Health Organization (WHO) criteria. Duration of response was

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TABLE 1.—Treatment results in relation to clinical features and to escalation levels of etoposide*

	Diagnosis										Escalation levels of etoposide		
	Morphology					Status							
	M1	M2	M3	M4	M5	Res	ER	R + Res	R + M	2nd R	3 × 100 mg/m²/day	4 × 100 mg/m²/day	5 × 100 mg/m²/day
Total	4	5	2	11	4	12	6	3	3	2	10	10	6
CR	0	2	0	4	3	3	3	0	2	1	2	4	3
PR	0	0	1	1	0	1	1	0	0	0	1	0	1

*Values = No. of patients. Res = primary resistance to TAD; ER = early relapse; R + Res = relapse and refractory; R + M = relapse under maintenance therapy; and 2nd R = 2nd or subsequent relapse.

counted from the day of diagnosis of CR to the day of bone marrow relapse.

RESULTS

Twenty-six patients were evaluable for response and toxicity. There were 18 males and eight females, with a median age of 47.0 years (range, 20–69). Other clinical data from these patients are summarized in table 1.

Twenty-one patients received one induction course and five received two induction courses. CR was achieved with one course in seven patients and with two courses in two patients. Higher response rates seemed to be achieved at higher dosages of etoposide (table 2). Two of the ten patients who received 3 × 100 mg/m²/day attained CR, whereas CR was achieved in four of ten (40%) who received 4 × 100 mg/m²/day and three of six (50%) who received 5 × 100 mg/m²/day. The median interval between initiation of therapy to diagnosis of CR was 40.0 days (range, 25–67). The median duration of CR was 85 days (range, 21–183+), excluding one patient who received autologous bone marrow transplantation.

The toxic effects in each escalation level are summarized in table 2. Minor toxic effects such as obstipation (grade 1–2 in four patients) and cardiotoxicity (tachycardia of 120/minute in one patient) are not shown. Fever of unknown origin occurred in 11 patients and infections occurred in ten patients. The infections included seven patients with pneumonia, two with septicemia, and one with periorbital phlegmon. Hematologic toxicity is expressed by the number of transfusions of erythrocytes (8.5 ± 5.3 units, mean ± SD) and platelets (5.7 ± 5.9 units) required per treatment course and by the duration of parenteral antimicrobial treatment (average, 15.6 ± 14.1 days). There was only one case of early death due to severe infection within the first 5 weeks of treatment.

DISCUSSION

In this phase I/II study, we have demonstrated that the combination of mitoxantrone and etoposide is active in the treatment of refractory leukemia. A total CR rate of 34% was achieved (90% confidence limits are 16%–49% for the true response rate).

As the toxic effects associated with this combination might be unpredictable, a careful dosage escalation of etoposide was done. Higher remission rates seemed to be attained in patients receiving 4–5 × 100 mg/m²/day of etoposide. Because the toxic effects observed at the dose level of 5 × 100 mg/m²/day were still acceptable and a considerable CR rate was achieved, we suggest that this is the optimal dose.

Other than nausea and stomatitis, the nonhematologic toxic effects were mild. However, severe aplasia was observed in all patients and was usually of long duration. In those patients who have responded, the mean time to recovery of granulocytes (> 1000/μl) and platelets (> 100,000/μl) was 37 days from initiation of therapy. The profound hematologic toxicity was reflected by the units of transfusions and the duration of parenteral antimicrobial treatment required per treatment course.

In conclusion, the present study shows that mitoxantrone combined with etoposide is effective in refractory

TABLE 2.—Nonhematologic and hematologic toxic effects in each escalation level

Escalation levels of etoposide	No. of treatment courses	Toxic effects by WHO grade*															Transfusions		Days of iv antibiotic therapy
		Nausea			Stomatitis			Diarrhea			Hepatotoxicity			Infection/FUO			No. of units of —		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Erythrocytes	Thrombocytes	
100 mg/m² × 3	10	3	1	0	2	3	0	1	2	0	1	0	0	0	0	5	10.0	4.0	11.5
100 mg/m² × 4	13	2	2	4	3	1	2	0	3	0	4	0	0	0	0	9	6.9	3.5	15.0
100 mg/m² × 5	8	4	4	1	1	4	1	0	3	1	2	1	0	0	0	7	5.7	6.0	18.0

*Values = No. of patients. FUO = fever of unknown origin. Grade 4 toxicity was observed in 1 patient with uncontrollable infection. Hematologic toxicity was expressed by the average No. of transfusions and by the duration of parenteral antibiotic treatment required during 1 course.

AML at an acceptable risk of toxicity. One-third of the patients with resistant AML achieved complete remission. It is possible that if this regimen is used in primary treatment, for either induction or consolidation, an improved remission rate or prolonged remission duration would result.

REFERENCES

1. GALE RP, FOON KA, and CLINE MJ. Intensive chemotherapy for acute myelogenous leukemia. Ann Intern Med 94:753–757, 1981.
2. PREISLER HD, BRECHER M, BROWMAN G, ET AL. The treatment of myelocytic leukemia in patients 30 years of age and younger. Am J Hematol 13:189–198, 1982.
3. WEINSTEIN HJ, MAYER RK, ROSENTHAL DS, ET AL. Treatment of acute myelogenous leukemia in children and adults. N Engl J Med 303:473–478, 1980.
4. PACIUCCI PA, OHNUMA T, CUTTNER J, ET AL. Mitoxantrone in patients with acute leukemia in relapse. Cancer Res 43:3919–3922, 1983.
5. ESTEY EH, KEATING MJ, MCCREDIE KB, ET AL. Phase II trial of mitoxantrone in refractory acute leukemia. Cancer Treat Rep 67: 389–390, 1983.
6. PRENTICE HG, ROBBINS G, MA DDF, ET AL. Mitoxantrone in relapsed and refractory acute leukemia. Semin Oncol 11(suppl 1):32–35, 1984.
7. MEYER P, HO AD, EHNINGER G, ET AL. Mitoxantrone in the treatment of relapsed and refractory acute leukemia. Invest New Drugs 3:203–206, 1985.
8. BENNETT JM, LYMANN GG, and CASSILETH PA. A phase II trial of VP-16-213 in adults with refractory acute myeloid leukemia. Am J Clin Oncol 7:471–473, 1984.
9. O'DWYER PJ, LEYLAND-JONES B, ALONSO MT, ET AL. Etoposide (VP-16-213)—current status of an active anticancer drug. N Engl J Med 312:692–700, 1985.

Phase II Study of Mitoxantrone in Patients With Mesothelioma: A National Cancer Institute of Canada Clinical Trials Group Study[1,2]

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The treatment of malignant mesothelioma with chemotherapeutic agents is disappointing. Doxorubicin, which is considered to be the most active single agent used to treat the disease, produces response rates ranging from 14% to 44% (1). Combination regimens incorporating doxorubicin offer little, if any, improvement on single-agent results, and the prognosis for advanced disease remains poor (2,3). Mitoxantrone is an anthraquinone derivative which, like doxorubicin, acts as a DNA intercalator but shows less preclinical cardiotoxicity (4,5). The National Cancer Institute of Canada Clinical Trials Group has recently completed a phase II study of this agent in previously untreated patients with mesothelioma. The results of that trial are the subject of this report.

Between July 1983 and February 1985, 24 patients from nine Canadian centers and six patients from an Australian center were entered in the trial. Eligibility criteria included: histologically confirmed malignant mesothelioma; no prior chemotherapy; Eastern Cooperative Oncology Group (ECOG) performance status < 3; radiographically and/or clinically measurable disease; serum creatinine ≤ 1.5 mg/dl; serum bilirubin ≤ 1.5 mg/dl; granulocyte count $\geq 2000/mm^3$; platelet count $\geq 100{,}000/mm^3$; and no organic heart disease. The Canadian Tumor Reference Centre reviewed pathology material on all patients from Canadian centers. Mitoxantrone was given as iv bolus injection every 3–4 weeks. The starting dose was 12 mg/m^2 and this was decreased on the basis of granulocytopenia or thrombocytopenia on the day of treatment. Doses were to be escalated by 2 mg/m^2/course until a granulocyte count nadir of $750/mm^3$ was achieved.

Complete response was defined as the disappearance of all evidence of active tumor for a minimum of 4 weeks. *Partial remission* was defined as a $\geq 50\%$ decrease in the sum of the products of measured lesions of at least 4 weeks' duration. *Progressive disease* was defined as a $\geq 25\%$ increase in the size of any lesion or the appearance of any new lesions. Patients were designated as having *stable disease* if their disease regressed $< 50\%$ or increased $< 25\%$ for a minimum duration of 8 weeks. All x-rays and computerized tomographic scans from Canadian centers were reviewed by a reference radiologist to assess response.

The characteristics of the 29 eligible patients entered on study are shown in table 1. Twenty-eight patients were evaluable for toxic effects and response (table 2). Nausea and/or vomiting was seen in 19 patients and was mild or moderate in degree. Granulocytopenia of < 1000 cells/mm^3 was documented in ten patients. Dose escalation was carried out in 17 patients, and four patients received doses ≥ 16 mg/m^2/cycle. Nonetheless, only four patients achieved granulocyte nadir counts of $< 750/mm^3$.

Two responses (one complete, 8 months' duration; one partial, > 2 months' duration) were seen in the 28 evaluable patients. This observation allows us to reject that the true response rate of mitoxantrone used in this dose and schedule is $> 21\%$ with a probability of error of < 0.05. While it is possible that a more intensive dosing schedule than that employed in this trial might

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	30
Ineligible	1*
Inevaluable	1†
Evaluable	28
Median age in yrs (range): 66 (33–75)	
Performance status (ECOG)	
0	2
1	16
2	11
Sites of disease at entry	
Pleura	29
Abdomen	0
Prior chemotherapy	0
Previous asbestos exposure	21
No. of cycles of mitoxantrone	
1	4
2	4
≥ 3	21

*Pathology review.
†Died on Day 4.

have yielded higher response rates, we feel that the limited activity of mitoxantrone observed in this previously untreated group of patients makes it unlikely that the drug will contribute significantly to the management of this disease.

TABLE 2.—Toxic effects and response*

	No. of patients
Toxic effect	
Nausea and vomiting	19
Hair loss	8†
Granulocytopenia (cells/mm^3)	
< 1500	16
< 1000	10
Thrombocytopenia (cells/mm^3)	
< 100,000	0
Infection/febrile neutropenia	2
Bleeding	0
Reponse	
Complete	1
Partial	1
Stable disease	15
Progressive disease	11

*28 evaluable patients.
†Mild in all patients.

REFERENCES

1. AISNER J, and WIERNIK P. Chemotherapy in the treatment of malignant mesothelioma. Semin Oncol 8:335, 1981.
2. CHAHINIAN AP, HOLLAND JF, and MANDEL EM. Chemotherapy for malignant mesothelioma with adriamycin and continuous infusion of 5-azacytidine. Cancer Treat Rep 62:1103–1109, 1978.
3. ANTMAN KH, BLUM RH, GREENBEGER JS, ET AL. Multimodality therapy for malignant mesothelioma based on a study of natural history. Am J Med 68:356–361, 1980.
4. HENDERSON BM, DOUGHERTY WJ, JAMES VC, ET AL. Safety assessment of a new anticancer compound, mitoxantrone, in beagle dogs: comparison with doxorubicin. I. Clinical observations. Cancer Treat Rep 66:1139–1143, 1982.
5. SPARANO BM, GORDON G, HALL C, ET AL. Safety assessment of a new anticancer compound, mitoxantrone, in beagle dogs: comparison with doxorubicin. II. Histologic and ultrastructural pathology. Cancer Treat Rep 66:1145–1158, 1982.

Phase II Evaluation of Acivicin in Lung Cancer: A Southeastern Cancer Study Group Trial[1,2]

Barnett S. Kramer,[2,*] Robert Birch,[2] Anthony Greco,[2] Karen Prestridge,[2] and Randall Johnson[3]

Acivicin bears structural similarity to L-glutamine and is an inhibitor of the four aminotransferases of the de novo purine and pyrimidine synthetic pathways, particularly CTP and GMP synthetase (1). Interference with the neurotransmitter glutamate recycling may mediate the CNS toxic effects of acivicin (2). Preclinical screening tests of acivicin demonstrated activity against human lung cancer xenografts (3). The recommended phase II dose for a daily × 5 schedule was 9-15 mg/m^2/day (4).

We performed a phase II study in patients with bronchogenic carcinoma who fulfilled the following criteria: histologically confirmed diagnosis; at least one evaluable lesion; Karnofsky performance status ≥ 60%; incurability by surgery or irradiation; relapse after prior treatment with first-line therapy in patients with small cell carcinoma; recovery from previous myelosuppressive therapy; no brain metastases or neuropsychiatric disease; and signed informed consent. Acivicin was administered at a dose of 12 mg/m^2 as an iv bolus infusion in normal saline on each of 5 successive days at 21-day intervals. The initial goal for accrual was 25 patients with each cell type, but this was not achieved in small cell or in the infrequent large cell type.

Eighty-six patients were entered, with a median Karnofsky performance status of 70% (range, 60%-100%) and a median age of 58 years (range, 36-83). Six patients were inevaluable: two were lost to follow-up before any assessment could be made and four had no follow-up data supplied. Of the remaining 80 patients, seven had disease limited to the chest and 73 had more extensive disease. Median numbers of prior chemotherapy courses were as follows: small cell carcinoma, two (range, one to four); and non-small cell carcinoma, one (range, zero to five). There were three partial responses (95% confidence intervals, 1%-11%) (table 1): two responses among 36 patients with adenocarcinoma and one response among 30 patients with squamous carcinoma. No responders had prior irradiation or chemotherapy. Responses lasted 9.5, 46, and 21 weeks. Overall median survival was 17 weeks for all eligible patients.

Toxic effects were considerable. There were six early deaths, none attributed to toxic effects: three of intercurrent disease and three of unknown cause. Drug-related toxic effects were primarily hematologic and neurologic (table 2). Neurotoxic effects were the most bothersome and dose-limiting.

We conclude that there was no significant activity of acivicin against bronchogenic carcinoma in a 12-mg/m^2 daily × 5 schedule every 3 weeks. The schedule does, however, produce significant neurotoxic effects.

TABLE 1.—Response*

Disease	No prior chemotherapy	Prior chemotherapy
Small cell carcinoma	—	0/7 (0%-44%)
Adenocarcinoma	2/18 (2%-36%)	0/18 (0%-22%)
Squamous carcinoma	1/17 (1%-31%)	0/13 (0%-28%)
Large cell carcinoma	0/1	0/6 (0%-48%)
Total	3/36 (2%-24%)	0/44 (0%-10%)

*Values = No. of patients with partial response/total No. of patients; Nos. in parentheses = 95% confidence intervals.

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TABLE 2.—Major toxic effects of acivicin

Toxic effect	% toxicity	
	No prior chemotherapy	Prior chemotherapy
Nadir granulocyte count ($\times 10^3$ cells/mm^3)		
1.5–1.89	3	13
1.0–1.49	9	3
0.5–0.99	6	8
< 0.5	3	3
Nadir platelet count ($\times 10^3$ cells/mm^3)		
90–129	6	5
50–89	—	5
25–49	—	—
< 25	—	5
Level of consciousness		
Transient lethargy	41	17
Somnolence	6	2
Obtunded	—	2
Coma	—	15
Ideation		
Intermittent confusion	24	12
Continuous confusion	6	2
Hallucination	—	15
Motor activity		
Mild hyperactivity	3	2
Tremor	—	2
Convulsions	3	—
Status epilepticus	—	—

REFERENCES

1. O'DWYER PJ, ALONSO MT, and LEYLAND-JONES B. Acivicin: a new glutamine antagonist in clinical trials. Am J Clin Oncol 2:1064–1071, 1984.
2. HERTZ L. Astrocytes. *In* Handbook of Neurochemistry, 2nd ed, Vol I: Chemical and Cellular Architecture (Lajtha A, ed). New York, Plenum Press, pp 484–511, 1982.
3. HOUCHENS DP, OVEJERA AA, SHERIDAN MA, ET AL. Therapy for mouse tumors and human tumor xenografts with the antitumor antibiotic AT-125. Cancer Treat Rep 63:473–476, 1979.
4. SRIDHAR KS, OHNUMA T, CHAHINIAN AP, ET AL. Phase I study of acivicin in patients with advanced cancer. Cancer Treat Rep 67:701–703, 1983.

Management of Advanced, Resistant Hodgkin's Disease and Non-Hodgkin's Lymphoma: A Phase II Study[1,2]

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In the malignant lymphomas, both Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL), complete responses (CRs) with initial radiotherapy or combination chemotherapy are frequently achieved. Although a significant proportion of patients will experience a long CR and subsequent cure, many will relapse or become resistant to initial therapy. Second-line or salvage regimens for these patients have yielded unsatisfactory results. We have investigated a program consisting of five cytotoxic drugs in an attempt to improve on these results.

The program consisted of cisplatin (P), etoposide (VP-16) (E), bleomycin (B), lomustine (CCNU) or carmustine (BCNU), and methotrexate (MTX) with leucovorin rescue [PEB/CCNU (BCNU), MTX-L].

PATIENTS AND METHODS

Twenty-five patients with advanced HD and NHL were treated with the PEB/CCNU (BCNU), MTX-L regimen. Patient characteristics are shown in table 1. The PEB/CCNU (BCNU), MTX-L regimen consisted of cisplatin (100 mg/m^2 iv on Day 1), etopside (100 mg/m^2 iv on Days 1, 3, and 5), and bleomycin (10 mg/m^2/day by continuous iv infusion on Days 1–4). Administration of PEB was repeated on Day 21. On Day 42, patients were treated with either CCNU (100 mg/m^2 orally) for HD or BCNU (200 mg/m^2 iv) for NHL, in addition to MTX (120 mg/m^2 iv on Days 42, 49, 57, and 65) followed by leucovorin rescue orally. A complete cycle was 84 days. If patients received more than one previous combination chemotherapy regimen and/or previous radiotherapy, the initial dose of VP-16 was decreased by 50%. After one complete 84-day cycle, patients were evaluated for response. Those patients showing any response or stable disease were maintained on therapy by initiating another cycle of PEB/CCNU (BCNU), MTX-L. Standard criteria were used to assess response.

RESULTS

Of the 25 patients started on the PEB/CCNU (BCNU), MTX-L regimen, 15 have completed at least one full 84-day cycle and are thus evaluable for response. All patients were evaluable for toxic effects. All patients had received prior therapy; 16 had received two or fewer regimens, and nine had received three or more regimens. One patient with HD who received more than three prior chemotherapy courses achieved a CR and is alive 12 months later. Three patients, all with NHL, achieved partial response; of these, one died of progressive disease, one has not received therapy for 18 months, and the third is currently receiving therapy for his disease. Five additional patients have stable disease. The overall response rate was 27% (the 95% interval around this response is very broad at 3.9%–49.5%). There are more patients with no response (62.5% vs 14%) in the group having received three or more prior chemotherapy regimens as compared to the group receiving two or fewer regimens. The median survival of the 15 evaluable patients has not yet been reached and is > 17 months.

The major toxic effects observed in our study were myelosuppression, infection, and pulmonary injury (table 1). Three patients had bacteremia during granulocyte count nadir and subsequently died from sepsis. Pulmonary function tests suggested pulmonary injury in six patients who had suggestive symptoms and radiographic abnormalities. This was documented by lung biopsy in two of these patients. Other toxic effects are shown in table 1.

DISCUSSION

Several previous reports of salvage therapy for resistant or relapsed lymphomas have yielded response rates of 30%–50% (1–3). Phase II trials with cisplatin have

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TABLE 1.—Patient characteristics and treatment results*

	1–2 prior chemotherapy courses	≥ 3 prior chemotherapy courses
Total	16	9
Evaluable	7	8
Median age in yrs (range)	41 (23–70)	37 (23–68)
Prior radiotherapy	5	7
Responses to primary regimen	5	4
Resistance to primary regimen	11	5
Histology		
HD	2	5
NHL	14	4
Median No. of PEB courses (range)†	1 (1/2–3)	1½ (1/2–3)
Toxic effects		
Nausea/vomiting	14 (87%)	8 (88%)
Platelet count < 100,000 cells/mm^3	13 (81%)	8 (88%)
Wbc count < 1500 cells/mm^3	10 (63%)	6 (66%)
Pulmonary	3 (19%)	3 (33%)
Infection	3 (19%)	2 (22%)
Renal	3 (19%)	2 (22%)
Death	1 (6%)	2 (22%)
Response		
CR	0 (0%)	1/8 (12.5%)
Partial response	2/7 (29%)	1/8 (12.5%)
Stable disease	4/7 (57%)	1/8 (12.5%)
None	1/7 (14%)	5/8 (62.5%)

*Unless otherwise specified, values = No. of patients.
†PEB = cisplatin, VP-16, and bleomycin.

demonstrated its activity against malignant lymphomas (4). Single-agent VP-16 therapy in phase II trials has also yielded partial response rates of 15%–30% (5). The combinations of cisplatin and VP-16 have also been studied with response rates up to 50% but with no documented CRs (5). Our results with this regimen do not seem to be more efficacious than single-agent VP-16 or combinations of VP-16 and cisplatin. While the agents in this regimen have activity against refractory lymphomas in heavily pretreated patients, the toxic effects are significant. We conclude that for the benefit achieved, the cost in terms of toxic effects is too high to employ the current regimen as salvage for patients with relapsed disease.

REFERENCES

1. LIEPMAN MK, WHEELER BH, ZUCKERMAN KS, ET AL. Combination chemotherapy of refractory lymphoma with *cis*-dichlorodiamineplatinum, vinblastine and bleomycin. Cancer 50:2736–2739, 1982.
2. CORDER MP, and CLAUSON GH. Salvage therapy of aggressive non-Hodgkin's lymphoma with a combination of vinblastine, bleomycin and *cis*-platinum. Cancer 54:202–206, 1984.
3. JUDSON IR, and WILTSHAW E. *cis*-Dichlorodiamineplatinum (*cis*-platinum) and etoposide (VP-16) in malignant lymphoma: an effective salvage regimen. Cancer Chemother Pharmacol 14:258–261, 1985.
4. CAVALLI F, JUNGI WF, NISSEN NI, ET AL. Phase II trial of *cis*-dichlorodiamineplatinum(II) in advanced malignant lymphoma. Cancer 48:1927–1930, 1981.
5. KRONER T, OBECHT JP, and JUNGI WF. Etoposide as single agent and in combination with *cis*-platinum for malignant lymphomas. Cancer Treat Rev 9(suppl A):39–43, 1982.

Phase II Trial of Epirubicin in Hepatoma[1]

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Hepatocellular carcinoma is now the second most common cause of cancer death in Hong Kong. The reported median survival ranges from 6 to 40 weeks (1-3). Most cases are inoperable at presentation (4). Radiotherapy has been shown to be ineffective, and various chemotherapeutic agents have been used with no major impact on survival. The most active agent reported so far is doxorubicin. This paper describes the results of a phase II study of epirubicin (a new analogue of doxorubicin) in hepatoma.

PATIENTS AND METHODS

Eligibility criteria for entry into the study were: clinical evidence of hepatomegaly with either positive histology for hepatoma or elevated alpha fetoprotein of > 500 IU/ml, and bilirubin < 2.4 mg/dl. The patients were given epirubicin, 75 mg/m^2 by iv bolus, every 3 weeks for a maximum of eight courses, if there was no evidence of disease progression. Since it was the first time the drug had ever been used in Chinese patients, it was considered safe to reduce the dose to 40 mg/m^2 in patients with bilirubin > 1.2 mg/dl. *Complete response* (*CR*) was defined as the complete disappearance of all clinical evidence of tumor. *Partial response* (*PR*) was defined as a $> 50\%$ reduction in the sum of the liver enlargement below the costal margin at midclavicular line and xiphoid process. *Disease progression* was defined as a $> 25\%$ increase in the sum of the liver enlargement below the costal margin at midclavicular line and xiphoid process. *Survival interval* was defined as the interval from the time of treatment to the time of death.

RESULTS

Seventeen patients were entered into the study, but only 14 patients were evaluable for response because of three early deaths. Nine of 14 patients had had prior surgical treatment. No patient had received any prior chemotherapy or radiotherapy. Their clinical characteristics and survival are shown in table 1. A total of 59 courses of epirubicin were administered. No response was seen in any of the 14 patients studied. Therefore, the true response rate was certainly $< 20\%$ at the 95% confidence interval (5). The treatment was fairly well tolerated; only two of the 14 patients (14%) developed grade 1 hematological toxicity (World Health Organization criteria), with a mild to moderate degree of nausea, vomiting, and alopecia. Pre- and post-treatment echocardiograms were performed in 50% of the patients and showed no significant drop in ejection fraction.

TABLE 1.—Patient characteristics and survival interval

Age in yrs	
Median	47.5
Range	36-70
Karnofsky performance status	
Median	70
Range	50-90
No. of courses given	
75 mg/m^2	
Median	5
Range	2-8
40 mg/m^2	
Median	3
Range	2-5
Bilirubin (mg/dl)	
Median	0.82
Range	0.23-2.11
Alpha fetoprotein (IU/ml)	
Median	4797.1
Range	10.9-235,200
Survival (days)	
Median	123
Range	35-304

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DISCUSSION

Our results are most disappointing. Although the true response rate must be < 20%, the median survival in our patients is still approximately 120 days. Therefore, the survival interval must be a measure of the success of the treatment in hepatocellular carcinoma. We conclude that epirubicin, in the present dose and form, has no effect on the tumor response and has no major impact on survival. It is relatively well tolerated, despite the altered liver function in many of our patients. Therefore, a higher dose of epirubicin is still worth exploring in a future trial.

REFERENCES

1. FALKSON G, MACINTYRE J, MOERTEL C, ET AL. Primary liver cancer: an Eastern Cooperative Oncology Group trial. Cancer 54: 970–977, 1984.
2. LIVER CANCER STUDY GROUP OF JAPAN. Primary liver cancer in Japan. Cancer 54:1747–1755, 1984.
3. CHILEBOWSKI R, TONG M, WEISSMAN J, ET AL. Hepatocellular carcinoma; diagnostic and prognostic features in North American patients. Cancer 53:2701–2706, 1984.
4. LIVER CANCER STUDY GROUP OF JAPAN. Primary liver cancers in Japan. Cancer 45:2663–2669, 1980.
5. LEE YJ, STAQUET M, and SIMON R. Two-stage plans for patient accrual in phase II cancer clinical trials. Cancer Treat Rep 63:1721–1726, 1979.

Phase II Trial of Bisantrene in Patients With Metastatic Melanoma[1,2]

Cindy Mackel, Frank L. Meyskens, Jr, and David S. Alberts[3,*]

Bisantrene is a new anthracene compound with clinical activity in the treatment of advanced breast cancer (1,2).

In vitro studies using clonogenic assays have shown significant cytotoxic effects with bisantrene against fresh human melanoma samples (3,4). Furthermore, in a phase I trial one patient with metastatic melanoma achieved a complete response lasting 6 months while receiving weekly bisantrene therapy (5). We have carried out a phase II trial of bisantrene, using a weekly dosing schedule, to determine the clinical activity level of this drug in the treatment of metastatic melanoma.

PATIENTS AND METHODS

Sixteen patients with biopsy-proven metastatic melanoma were entered in this phase II study. All patients had measurable disease, life expectancy of at least 12 weeks, and Eastern Cooperative Oncology Group performance status ≤ 2. Nine of the 16 patients (56%) had had no prior chemotherapy and five (31%) had received treatment with only one cytotoxic agent.

Bisantrene was diluted in 500 ml of 5% dextrose in water. The drug was infused over 2 hours through a centrally located Silastic catheter or a peripheral vein. Hydrocortisone (100 mg) and diphenhydramine hydrochloride (50 mg) were given by bolus iv injection just prior to each bisantrene dose in an attempt to prevent anaphylactoid-type reactions. The starting dose of bisantrene was 200 mg/m^2/week for 3 consecutive weeks, based on a phase I dosing study by Alberts et al (5). The drug was administered as a single weekly dose for 3 consecutive weeks with a 2-week drug-free interval between courses.

RESULTS

Sixteen patients were entered in the phase II trial over a 30-month period. There were 11 males and five females ranging in age from 24 to 71 years (median, 50). One hundred twenty-five doses of bisantrene were administered to 16 patients (range, one to 39). Three patients received lowered doses after their first course of drug administration (range, 8%–20% decrease). None of the 16 patients experienced an objective response to bisantrene treatment; however, two patients did achieve long-term (12 and 21 months) disease stabilization while continuing to receive bisantrene therapy.

Bisantrene was tolerated relatively well by this patient population. Leukopenia was the dose-limiting toxic effect of the drug. Moderate (wbc count, 2000–2999/μl) to severe (wbc count 1000–1999/μl) leukopenia after two consecutive doses occurred in 11 of 16 patients (69%) during their first course of bisantrene. Three additional patients experienced moderate to severe leukopenia after two weekly doses during their second course. There was one treatment-related death associated with severe drug-induced leukopenia. The nadir wbc count was 1300/μl and the nadir absolute polymorphonuclear neutrophil count was 1079/μl. The patient died of fulminant pneumonia and sepsis.

Mild (hemoglobin, 9.0–9.9 g/dl) to moderate (hemoglobin, 7.0–8.9 g/dl) anemia occurred in two patients after the first dose of their second therapy course. Two patients experienced mild hypotension (ie, decreases of four to 14 mm Hg diastolic pressure) during drug infusion. Mild nausea and vomiting, moderate fever, sweats and diarrhea, and significant increase in arthritic pain each occurred in a single patient. Mild alopecia ($< 25\%$ hair loss) was seen in only one patient.

DISCUSSION

Bisantrene was selected for treatment of metastatic melanoma because of its significant in vitro activity against fresh human melanoma samples in the human tumor cloning assay (3,4) and the long-term complete response observed in one melanoma patient entered in a phase I trial (5). Unfortunately, in the present trial bisantrene failed to demonstrate significant antitumor activity in 14 of 16 patients with melanoma. Two

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patients, who did experience long-term (12 and 21 months) stable disease, received high cumulative doses of bisantrene, but the standard criteria for objective response were not met in these patients. Spiegel et al (6) have also failed to observe significant activity for bisantrene in patients with metastatic melanoma.

Although bisantrene does not appear to be a clinically useful drug for the treatment of melanoma, it is active in patients with advanced breast cancer (1,2). The relative lack of chronic toxic effects associated with long-term bisantrene therapy in this and other trials (1,2) suggests that it will be an unusually well-tolerated drug in the breast cancer patient.

Although bisantrene causes local soft tissue toxic effects at the site of administration in a high percentage of patients (5), the use of a subclavian vein catheter in patients entered in this trial completely eradicated this problem. Leukopenia was dose-limiting, but was rarely life-threatening and was always of short duration. It is of considerable interest that by using a weekly dose schedule of 200 mg/m^2 for 3 consecutive weeks of each month it was possible to administer almost twice the routinely used once every 3 week dose of 260 mg/m^2 (7). Consideration should be given to the use of this weekly dose schedule in future trials of bisantrene in the treatment of breast cancer.

REFERENCES

1. YAP H, YAP B, BLUMENSCHEIN GR, ET AL. Bisantrene, an active new drug in the treatment of metastatic breast cancer. Cancer Res 43:1402–1404, 1983.
2. OSBORN CK, VON HOFF DD, COWAN JD, ET AL. Bisantrene, an active drug in patients with advanced breast cancer. Cancer Treat Rep 68:357–360, 1984.
3. VON HOFF DD, FORSEITH B, METELMANN H, ET AL. Direct cloning of human malignant melanoma in soft agar culture. Cancer 50:696–701, 1982.
4. SALMON SE, MEYSKENS FL, JR, ALBERTS DS, ET AL. New drugs in ovarian cancer and malignant melanoma: in vitro phase II screening with the human tumor stem cell assay. Cancer Treat Rep 65:1–12, 1981.
5. ALBERTS DS, MACKEL C, POCELINKO R, ET AL. Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-*H*-imidazol-2-yl)hydrazone] dihydrochloride with correlative in vitro human tumor clonogenic assay. Cancer Res 42:1170–1175, 1982.
6. SPIEGEL RJ, LEVIN M, CUMPS E, ET AL. Phase II trial of bisantrene in melanoma with clonogenic assay correlations. Proc ASCO 1:175, 1982.
7. VON HOFF DD, MYERS JW, KUHN J, ET AL. Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis [(4,5-dihydro-1*H*-imidazol-2-yl) hydrazone] dihydrochloride (Cl 216,942). Cancer Res 41:3118–3121, 1981.

Phase II Trial of Baker's Antifol in Patients With Recurrent or Inoperable Head and Neck Cancer[1,2]

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Baker's antifol (triazinate) is an antifol with a different toxicity spectrum than that of methotrexate. We administered triazinate by a 1-hour iv infusion to 29 ambulatory, consenting patients with advanced and/or recurrent squamous cell head and neck cancer with measurable or evaluable disease. The dose was 300 mg/m^2/week, increasing by 100 mg/m^2/week to a maximum of 600 mg/m^2/week. Tumor response and toxic effects were assessed weekly according to standard criteria (1). Doses were reduced or delayed for hematologic toxic effects or for mucositis and stopped permanently for generalized dermatitis. Neurologic toxic effects were treated by lengthening the infusion time.

Patient characteristics are shown in table 1. Twenty-three patients reached a dose of at least 500 mg/m^2, that used in prior trials on weekly schedules (2,3).

Four patients developed transient erythema multiforme and four had minor skin reactions. One patient developed transient bronchospasm after six doses. Most patients developed hyperpigmented skin over infused veins, a phenomenon we previously reported in detail (4). Transient neurologic toxic effects (tremors, dizziness, paresthesias, or visual disturbances) occurred in six patients. An additional patient had a seizure 1 week after his third dose that was probably not treatment-related. One patient had respiratory arrest after inadvertently receiving approximately 400 mg of triazinate over 5 minutes but was resuscitated without sequelae. Leukopenia (range 2400–3700 cells/mm^3) and one instance of thrombocytopenia (67,000 cells/mm^3) occurred among four patients. One patient each had grade 1 mucositis and grade 1 diarrhea; five had mild to moderate nausea.

There were two partial responders among 27 evaluable patients (true response rate < 17.5%, with 95% confidence limits). One responder developed bronchospasm after his sixth treatment and relapsed 2 weeks later. The other patient became lost to follow-up, returned after 7 months, and achieved a second response lasting 5 weeks.

Although heavily pretreated, most patients had a good performance status. Furthermore, there was only one responder among 18 patients with no prior chemotherapy. We conclude that weekly iv triazinate is inactive in this patient population.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 60 (40–83)	
Sex	
Male	24
Female	5
Performance status	
0–1	16
2	8
3	5
Previous treatment	
Surgery only	1
Radiotherapy only	7
Chemotherapy only	2
Surgery + radiotherapy	7
Surgery, radiotherapy, + chemotherapy	9
None	3
Tumor sites	
Oral cavity	6
Oropharynx	11
Hypopharynx/larynx	7
Nasopharynx	3
Unknown	2

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REFERENCES

1. KRASNOW SH, COHEN MH, JOHNSTON-EARLY A, ET AL. Combined therapy for stage III–IV head and neck cancer: preliminary results. J Clin Oncol 2:804–810, 1984.
2. CREECH RH, MEHTA CR, COHEN M, ET AL. Results of a phase II protocol for evaluation of new chemotherapeutic regimens in patients with inoperable non-small cell lung carcinoma. Cancer Treat Rep 65:431–438, 1981.
3. ARSENEAU JC, BUNDY B, DOLAN T, ET AL. Phase II study of Baker's antifol (triazinate, TZT, NSC-139,105) in advanced squamous cell carcinoma of cervix. Am J Clin Oncol 5:61–64, 1982.
4. KRASNOW SH, PATTERSON RH, FISCHMANN B, ET AL. Local toxicity from iv administration of Baker's antifol. Cancer Treat Rep 69:735–736, 1985.

Phase II Trial of Mitoguazone in Children With Leukemia and Lymphoma: A Pediatric Oncology Group Study[1,2]

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Mitoguazone (MGBG) has activity in acute nonlymphocytic leukemia (ANLL) and lymphoma. Daily administration produces severe toxic effects, possibly related to slow excretion (1-3). Weekly MGBG (500 mg/m^2), however, is well-tolerated (4).

The Pediatric Oncology Group (POG) evaluated MGBG in relapsed ANLL, acute lymphocytic leukemia (ALL), chronic myelogenous leukemia in blast crises (CML), and non-Hodgkin's lymphoma (NHL). Informed consent, bilirubin < 2 mg/dl, and either a creatinine < 1.2 mg/dl or BUN < 20 mg/dl were required prior to treatment. The initial MGBG dose was 400 mg/m^2, 20% below that tolerated by adults with advanced cancer (4). Subsequent doses, administered weekly, were escalated by 100 mg/m^2/dose. If toxic effects occurred, the next dose, reduced by 100 or 200 mg/m^2, was administered after toxic effects were resolved. Diluted in either normal saline or 5% dextrose in water, MGBG was infused iv over 30-60 minutes. Response and toxic effects were scored by POG criteria. MGBG was discontinued if no response occurred after four doses and sooner if the disease rapidly progressed. Concomitant intrathecal chemotherapy was given to nine patients.

No complete remissions were attributed solely to MGBG (table 1). One ALL patient with a remission received daily prednisone for presumed adrenal insufficiency after the third dose. This patient had three prior remissions and three previous courses of steroids. MGBG was administered once every 2 weeks during the 12-week remission.

TABLE 1.—Clinical data*

	No. of patients	Average No. of prior inductions/patient	No. of patients with — Complete response	Partial response	Progressive disease	Early death	No. of refusals
≥ 4 MGBG doses							
ALL	9	3.3	1†	0	8	NA	0
ANLL	14	3.1	0	1‡	13	NA	0
NHL	1	NA	0	0	1	NA	0
< 4 MGBG doses							
ALL	23§	4.1	0	0	7	11	4
ANLL	8	2.8	0	0	3	5	0
NHL	1	NA	0	0	0	1	0
CML	2	1.5	0	0	2	0	0

*NA = not applicable.
†Patient received concomitant prednisone.
‡Decrease in % of marrow blasts to 16% after 6 MGBG doses.
§1 patient had MGBG discontinued for severe stomatitis.

[1] Received July 15, 1985; revised Apr 3, 1986; accepted Apr 11, 1986.
[2] Supported in part by Public Health Service grants CA-15525, CA-29139, CA-13161, CA-33625, and CA-30969 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3] Pediatric Hematology-Oncology, Duke University Medical Center, Durham, NC.
[4] Statistical Office, College of Medicine, University of Florida, Gainesville.
[5] Baylor College of Medicine, Houston, TX.
[6] Emory University School of Medicine, Atlanta, GA.
[7] University of Texas Southwestern Medical School at Dallas.
[8] We thank Martha Timmons for secretarial assistance in the preparation of the manuscript.
* *Reprint requests to:* Thomas R. Kinney, MD (POG 8021), Pediatric Oncology Group, 2nd Floor, Suite A, 4949 W Pine Blvd, St. Louis, MO 63108.

TABLE 2.—Toxicity

Dose (mg/m^2)	No. of patients with —				No. of doses	Toxic effect*					
						Stomatitis		Diarrhea		Nausea and vomiting	
	ALL	ANLL	NHL	CML		Moderate	Severe	Moderate	Severe	Moderate	Severe
200	1	0	0	0	1	0	0	0	0	0	0
300	1	2	0	0	6	0	1	0	0	0	0
400	32	20	2	2	88	6	5	3	1	2	3
500	14	16	2	1	47	3	3	0	0	1	1
600	8	9	1	1	24	1	2	0	0	1	4
700	3	6	1	0	18	0	1	0	0	1	2
Totals					184	10	12	3	1	5	10

*Other toxic effects included painful nodular skin eruption (1 patient), myalgia (1 patient), significant weight loss (2 patients), and phlebitis (1 patient).

Neither hypoglycemia nor hepatic or renal toxic effects were observed. Transient allergic skin reactions were ameliorated on subsequent doses by steroids and diphenhydramine hydrochloride. Toxic effects are shown in table 2.

Therapeutic efficacy for MGBG was not demonstrated. The discrepancy between this and prior reports in ANLL may reflect less MGBG administered or refractory disease as evidenced by prior failures to multiple agents.

Weekly MGBG doses between 400 and 700 mg/m^2 were well-tolerated, useful data for future trials using larger MGBG doses or MGBG in combination with agents such as α-difluoromethylornithine (5).

REFERENCES

1. CARBONE PP, FREIREICH EJ, FREI E, III, ET AL. The effectiveness of methylglyoxal bis guanylhydrazone (CH_3-G) in human malignant diseases. Acta un Int Cancer 20:340–343, 1964.
2. LEVIN RH, HENDERSON E, KARON M, ET AL. Treatment of acute leukemia with methylglyoxal-bis-guanylhydrazone (methyl GAG). Clin Pharmacol Ther 6:31–42, 1965.
3. OLIVERIO VT, and ZUBROD CG. Clinical pharmacology of the effective anti-tumor drugs. Annu Rev Pharmacol 5:335–356, 1965.
4. KNIGHT WA, III, LIVINGSTON RB, FABIAN C, ET AL. Methylglyoxal bis-guanylhydrazone (methyl-Gag, MGBG) in advanced human malignancy. Proc Am Assoc Cancer Res and ASCO 20:319, 1979.
5. SIIMES M, SEPPANEN P, ALHONEN-HONGISTO L, ET AL. Synergistic action of two polyamine antimetabolites leads to a rapid therapeutic response in childhood leukemia. Int J Cancer 28:567–570, 1981.

Phase II Evaluation of Mitoxantrone in Advanced Carcinoma of the Stomach: A Southeastern Cancer Study Group Trial[1,2]

Philip A. DeSimone,[3,*] Richard Gams,[4] and Robert Birch[4]

Mitoxantrone is one of a group of bis-substituted anthraquinones that binds avidly to DNA and is highly active in animal tumor models (1,2). These properties led to its evaluation in the treatment of adenocarcinoma of the stomach.

PATIENTS AND METHODS

Consenting patients with histologically confirmed metastatic carcinoma of the stomach no longer treatable primarily with surgery and radiation therapy were eligible for this study. All patients must have had measurable disease, life expectancy of at least 6 weeks prior to starting chemotherapy, Karnofsky performance status > 60%, caloric intake > 2500 kcals (as assessed by actual caloric count), adequate bone marrow reserve with absolute granulocyte count > 2500/mm^3, platelet count > 125,000 mm^3, and bilirubin < 1.2 mg/100 ml. Patients who had received prior chemotherapy must not have received any agents within 4 weeks (7 for a nitrosourea). All patients were screened for coexistent heart disease or cardiac arrhythmia. Patients who had received doses of prior doxorubicin therapy had to have a normal cardiac ejection fraction.

Mitoxantrone at a dose of 5 mg/m^2 iv weekly × 6 was required for an adequate trial. This schedule was chosen by Lederle Laboratories as an alternative to the schedule used by Cowan et al (3). Patients with rapid disease progression after two weekly infusions could be removed from the study. Patients who achieved complete response or partial response or had stable disease with no hematologic or cardiotoxic effects after the six weekly injections were to be continued in the study and escalated to a dose of 6 mg/m^2. All toxic effects were graded according to current criteria (4).

RESULTS AND DISCUSSION

Thirty-four patients were entered in the study. Two patients were unevaluable because of a lack of measurable disease. Thirty-two patients were evaluable for toxic effects and response to chemotherapy (16 each in the no prior and the prior therapy groups). No patients were removed from the study. Demographic data for the patients studied are shown in table 1. Four patients (two in each group) had evidence of rapid disease progression and received only two courses of chemotherapy. Their data were used in the toxic effects evaluation.

The primary diagnosis of carcinoma of the stomach was proven by a surgical biopsy or inspection plus needle biopsy in all patients. The overall male to female ratio of 5.8:1 differs greatly from nationally reported 2:1 statistics (5). The primary site of measurable metastatic disease was hepatic or intra-abdominal in 85% of all patients. The major toxic effect was readily reversible absolute granulocytopenia in 59% of the patients. In patients receiving prior therapy, the toxic effects tended to be grade III, compared to grade II seen in

TABLE 1.—Patient characteristics*

	Prior therapy	No prior therapy
Total patients entered/evaluable	17/16	17/16
Median age in yrs (range)	57 (39–74)	57 (39–74)
Prior treatment	16	
1st site of metastatic disease		
Liver	12	9
Abdominal mass	2	6
Lung	2	
Toxic effect		
Absolute granulocyte count (× 10^3 cells/mm^3)		
Grade I (1.5 to < 1.9)	1	
Grade II (1.0 to < 1.5)	2	2
Grade III (0.5 to < 1.0)	5	2
Median survival in wks from start of study	7.7	10.6
Overall survival in wks	9.1	

*Unless otherwise specified, values = No. of patients.

[1] Received Aug 6, 1985; revised Feb 24, 1986; accepted Apr 11, 1986.
[2] Supported by Public Health Service grants CA-19657, CA-24456, CA-12223, CA-03013, CA-03177, CA-03227, CA-03376, CA-28169, CA-23909, CA-30247, CA-27587, CA-05461, CA-07961, CA-15584, and CA-20255 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Medical Service, Veterans Administration Medical Center, Lexington, KY.
[4] Southeastern Cooperative Cancer Study Group, University of Alabama, Birmingham.
Reprint requests to: Philip A. DeSimone, MD, Medical Service (111), Veterans Administration Medical Center, Lexington, KY 40511.

patients receiving no prior therapy. No cardiac toxic effect was noted. Only one patient had blue-green-colored urine.

No responders were noted in either treatment group in the study. It is our conclusion that the weekly schedule of mitoxantrone is not useful in the treatment of adenocarcinoma of the stomach.

REFERENCES

1. DOUBLE J, and BROWN JR. Evaluation of the binding of some substituted anthraquinones and naphthacenequinones to DNA. J Pharm Pharmacol 28:166–169, 1976.
2. JOHNSON RK, ZEE-CHENG RK-Y, LEE WW, ET AL. Experimental antitumor activity of aminoanthraquinones. Cancer Treat Rep 63:425–439, 1979.
3. COWAN JD, VON HOFF DD, MCDONALD B, ET AL. Phase II trials of mitoxantrone in previously untreated patients with colorectal adenocarcinoma: a Southwest Oncology Group Study. Cancer Treat Rep 66:1779–1780, 1982.
4. DESIMONE PA, GAMS R, and BARTOLUCCI A. Phase II studies of mitoxantrone (dihydroxyanthracenedione) in the treatment of advanced colorectal carcinoma. Am J Clin Oncol 7:517–522, 1984.
5. Estimated for new cancer cases by sex for all sites—US, 1985, CA 35:26, 1985.

ANNOUNCEMENTS

Association Opens Applications for 1987 Fellowships

The Association for Brain Tumor Research, Chicago, is now accepting applications for fellowships from postdoctoral candidates whose intent is to pursue a career in brain tumor research. Preference will be given to MD's who are U.S. citizens or permanent residents of the U.S., seeking research training that will enable them to pursue careers as clinical investigators.

AFBTR fellowships offer an award of $18,000 per annum plus a $2000 annual travel allowance for a period of two years, with a third-year renewable option. Each year's travel must include one presentation to the AFBTR Board of Directors in Chicago.

All applications will be reviewed by the Association's Medical Advisory Council and awards will be made based on their recommendations.

Criteria for selection will include the quality of the individual applicant; the quality of the program to which s/he is applying; and the research s/he is undertaking.

Applications must be submitted by the sponsoring program director, and deadline for completed applications, including letters of recommendation, is January 9, 1987. Awards will be announced by April 1, 1987, for fellowships to begin July 1, 1987.

Application forms may be obtained from the Association for Brain Tumor Research, Suite 200, 6232 N Pulaski Rd, Suite 200, Chicago, IL 60646, and completed applications should be mailed to the same address.

For further information, contact: the Association's Executive Director, Donald Segal (telephone: 312-782-3800).

International Congress on the Biological and Clinical Aspects of Phagocyte Function

Pavia, Italy, September 7–10, 1986

The Italian Society of Hematology is organizing the International Congress on the Biological and Clinical Aspects of Phagocyte Function to be held in Pavia, Italy, September 7–10, 1986. Topics for discussion include: leukocyte random locomotion, chemotaxis, and chemokinesis; membrane metabolism; pharmacological manipulation and immunomodulation of phagocyte function; genetic control of phagocyte function; the role of phagocytes in inflammation; leukotrienes, prostaglandins, chemotaxis, and phagocyte function; membrane chemotactic and phagocyte function; and the clinical aspects of phagocyte disorders.

The program will include free oral presentations and poster sessions selected from the submitted abstracts. Registration fee after May 31, 1986, is $200 US.

For further information, contact: Giovanni Ricevuti, Istituto di Patologia Speciale Medica 1ª, Università di Pavia, 27100 Pavia, Italy (telephone: 0382/390590).

Nutritional and Pharmacological Treatment of Brain Tumors

Pavia, Italy, September 18, 1986

The International Meeting on Nutritional and Pharmacological Treatment of Brain Tumors will be held in Pavia, Italy, on September 18, 1986, and is a Satellite Meeting of the XXXV Italian Congress of Neurosurgery.

For further information, contact: Prof. Rodolfo Paoletti, Centro Studi Dell'Alimentazione, via S. Pietro all'Orto, 17, 20121 Milan, Italy (telephone: 02/709.514).

35th National Meeting of the Italian Neurosurgical Society

Pavia, Italy, September 18-20, 1986

Topics of discussion at this meeting include cerebral aneurisms, pathology of the cervical column, brain tumors in infancy, spinal tumors, cerebral ischemia, and pain therapy.

For further information, contact: Prof. Pietro Paoletti, Department of Surgery, Neurosurgical Clinic, University of Pavia, San Matteo Hospital, 27100 Pavia, Italy (telephone: 0382/422231).

Diagnosis and Therapy of Bone Marrow Abnormalities: Cancer and Stem Cell Defects

San Francisco, California, September 25-26, 1986

The Bone Marow Transplant Unit of the Cancer Research Institute and the Department of Pediatrics of the University of California School of Medicine in San Francisco announce a symposium on diagnostic and therapeutic advances in bone marrow abnormalities. Designed for pediatric and general oncologists, the program will be held at the Sheraton on Fisherman's Wharf in San Francisco, California, September 25-26, 1986. Speakers include members of the Departments of Pediatrics and Medicine (Oncology) of the University of California School of Medicine as well as featured guest faculty. The program will meet the criteria for ACCME/AMA/CMA Category 1 credit.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California, San Francisco, CA 94143 (telephone: 415-476-4251).

Principles of Epidemiology

Cambridge, Massachusetts, October 6-8, 1986

The New England Epidemiology Institute will present a 3-day course on "Principles and Methods of Epidemiology," at the Charles Hotel in Cambridge, MA. The course will be taught by Drs. Kenneth Rothman and Stephen Lanes and by Mr. Charles Poole. The course will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. No previous study of epidemiology or biostatistics is required. Registrants may receive Continuing Medical Education Credits (AMA Category 1) upon application and/or Certification Maintenance Credits from the American Board of Industrial Hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Mechanisms of Drug Resistance in Neoplastic Cells

Washington, DC, October 15-16, 1986

The Ninth Annual Bristol-Myers Symposium on Cancer Research entitled "Mechanisms of Drug Resistance in Neoplastic Cells" will be held on October 15-16, 1986, at the Washington Hilton, Washington, DC. The meeting is being hosted by the Vincent T. Lombardi Cancer Research Center of Georgetown University. The registration fee is $75 ($20 for students, residents, and fellows). Preregistration is required.

For further information, contact: Mary Day, Office of Continuing Medical Education, Georgetown University Medical Center, 3800 Reservoir Rd, NW, Washington, DC 20007 (telephone: 202-625-2306).

Second International Symposium on Epstein-Barr Virus

St. Petersburg, Florida, October 17-22, 1986

This symposium, dedicated to the memory of Professor Yohie Ito, will cover the current advances in Epstein-Barr virus (EBV)-associated diseases such as Burkitt's lymphoma, nasopharyngeal carci-

noma, lymphoproliferative syndrome, chronic infectious mononucleosis, and immunodeficiency syndromes including AIDS and organ transplants. The various scientific presentations will include overviews, selected papers, and poster sessions. The program will concentrate on both clinical and basic research aspects. Specifically, the symposium will review the latest advances in disease manifestations associated with EBV infections and will cover clinico-pathologic features, epidemiologic and genetic aspects, and new developments in basic research on EBV including: intraction of oncogenes, EBV genomes, gene expressions, mapping and proteins, cellular immune responses, cofactors associated with EBV infections, and control of EBV infections.

Dr. M. Nonoyama, Showa University Research Institute for Biomedicine in Florida; Dr. R. Glaser, Ohio State University; Dr. G. Pearson, Georgetown University; and Drs. D. Ablashi and P. Levine, National Cancer Institute, Bethesda, MD, are organizing the symposium.

For further information, contact: Dr. Nonoyama, Showa University Research Institute for Biomedicine in Florida, St. Petersburg, FL 33702; or Dr. Ablashi, Bldg 37, Rm 1E24, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (telephone: 301-496-9332).

1st Certifying Examination of the American Board of Laser Surgery

New Orleans, Louisiana, October 18, 1986

The American Board of Laser Surgery announces its 1st Certifying Examination on Saturday, October 18, 1986, at Tulane University, New Orleans, Louisiana.

For further information, contact: Stanley Stellar, MD, St Barnabas Medical Center, Old Short Hills Rd, Livingston, NJ (telephone: 201-533-5070).

Fifth Annual Cincinnati Cancer Conference

Cincinnati, Ohio, October 31–November 1, 1986

Breast cancer and cancer nursing will be the focuses of the Fifth Annual Cincinnati Cancer Conference. Cancer specialists from across the country will discuss various modalities in the treatment of breast cancer, including surgery, radiotherapy, new directions in chemotherapy and hormonal therapy, and endocrine treatment. The detection of breast cancer and the role of diet in breast cancer will also be covered.

Sessions devoted to nursing issues will take up various topics, such as: legal and ethical issues in outpatient and home care; the creation of effective interdisciplinary relationships in outpatient and home care; implementation of research in pain control; advanced cancer nursing practice; and a 10-year cancer nursing forecast.

Conference coordinators are Barth Hoogstraten, MD, and Hilary Wood, RN. The conference is sponsored by Bethesda Hospital, Inc, of Cincinnati.

For further information, contact: Thomas O'Connor, Continuing Medical Education, Bethesda Hospital, Inc, 619 Oak St, Cincinnati, OH 45206.

12th Annual Congress of the European Society for Medical Oncology

Nice, France, November 28–30, 1986

The scientific program for the 12th Annual Congress of the European Society for Medical Oncology will include: 1. Reports on the most important 1985–1986 international meetings devoted to cancer. 2. Nine sessions with oral communications and posters on preclinical and clinical phase I–II studies, biological response modifiers, medical treatments, and therapeutic strategies. 3. Plenary lectures on medical oncology.

For further information, contact: Prof. M. Schneider, Centre Antoine-Lacassagne, 36 Voie Romaine, 06054 Nice Cedex, France (telephone: 93 81 71 33).

1987 UCLA Symposia on Cellular and Molecular Biology of Cancer

an Ortho-UCLA Symposium
Growth Regulation of Cancer
Organizer: Marc E. Lippman
Park City, Utah, January 17-23, 1987*

Steroid Hormone Action
Organizer: Gordon Ringold
Park City, Utah, January 17-23, 1987*

a Schering-UCLA Symposium
Recent Advances in Leukemia and Lymphoma
Organizers: Robert Peter Gale and David W. Golde
Keystone, Colorado, January 25-31, 1987*

an Abbott-UCLA Symposium
Human Retroviruses, Cancer and AIDS:
Approaches to Prevention and Therapy
Organizer: Dani Bolognesi
Keystone, Colorado, April 1-6, 1987**

a Triton Biosciences-Smith, Kline & French-UCLA Symposium
Tumor Progression and Metastasis
Organizers: Isaiah J. Fidler and Garth L. Nicolson
Keystone, Colorado, April 6-12, 1987**

a Smith Kline & French-Triton Biosciences-UCLA Symposium
Altered Glycosylation in Tumor Cells
Organizers: Sen-Itiroh Hakomori, Donald Marcus
and Christopher Reading
Keystone, Colorado, April 6-12, 1987**

Abstract submission deadlines are *October 3, 1986* (*) and *December 12, 1986* (**). Applications will be accepted at any time for meetings which are not over-subscribed.

For further information, contact: UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, CA 90024 [telephone: (213)-206-6292].

35th Annual Meeting of the Radiation Research Society

Atlanta, Georgia, February 22-26, 1987

For further information, contact: Radiation Research Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3153).

Grand Orient de Belgique Mac.·. Lodge Les Amis du Commerce et La Perseverance Reunis 4th Medical Prize

Antwerp, Belgium, March 1987

In March 1987, the Masonic Lodge "Les Amis du Commerce et la Perseverance Reunis" in Antwerp will award its 4th Medical Prize of 30,000 ECU [European Currency Units, 1 ECU ≈ 1 US dollar]. The purpose of the prize is to reward a scientist or group of scientists who, in the course of their research, have made a significant contribution to the progress of medical science whether theoretical or practical, fundamental, or clinical. The prize may be divided among several candidates.

The prize will be awarded by jury of four members and a president appointed by the Masonic Lodge "Les Amis du Commerce et la Perseverance Reunis." The jury shall be entitled to call in experts for advice if it deems necessary. The members of the jury who will have been empanelled to attend the meetings will have to justify their judgment of the candidacies in writing. All deliberations will be made in camera and decisions made by a simple majority. All working expenses will be paid by the organizing lodge.

The competition is open to any researcher whether certified or not, without discrimination of a racial, national, sexual, or philosophical nature. Candidacies for the prize must be sponsored by at least two Freemasons whose masonic qualifications must be confirmed by their Masonic Authorities. In turn the sponsors will have to vouch in writing for the moral integrity of their candidates.

All applications must be accompanied by a detailed curriculum vitae of the candidates, a resume of their scientific activities, and the opinion of the authorities under whom they work. All applications must be submitted before *December 30, 1986,* and addressed to: Mr. René De Zuttere, Hoogpadlaan 101, B-2070 Antwerp, Belgium.

The jury will come to a decision not later than March 31, 1987. The decision will be final and not open to appeal. The prize will be presented during an academic session in Antwerp in May 1987. The Masonic Lodge "Les Amis du Commerce et la Perseverance Reunis" reserves the right to withhold the prize should the applications appear to be below standard.

For further information, contact: Mr. René De Zuttere, Hoogpadlaan 101, B-2070 Antwerp, Belgium.

American Radium Society Sixty-Ninth Annual Meeting

London, England, April 6–10, 1987

The Portman Inter-Continental in London will be the site of the sixty-ninth annual meeting of the American Radium Society, April 6–10, 1987.

For further information, contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

International Conference on Reproduction and Human Cancer

Bethesda, Maryland, May 11–13, 1987

The National Cancer Institute and the National Institute of Child Health and Human Development are sponsoring an International Conference on Reproduction and Human Cancer to be held in Bethesda, MD, May 11–13, 1987, to focus on the effects of cancer and its treatment on reproduction, including possible genetic effects. Poster sessions are planned to accommodate offered papers.

For further information, contact: Drs. John J. Mulvihill (Landow Bldg, Rm 8C41) or Richard J. Sherins (Bldg 10, Rm 10N234), National Institutes of Health, Bethesda, MD 20892; or call Conference Manager, Ms. Mary E. Clark (telephone: 301-589-6760).

CORRECTION

In the article "Neurotoxicity and Efficacy of Combined Vinca Alkaloids in Breast Cancer" by D. J. Stewart, J. A. Maroun, B. Lefebvre, and R. Heringer (Cancer Treat Rep 70:571–573, May 1986), line 6 (column 1) on page 573 should read ". . .using vinblastine doses as high as 6 mg/m^2," (*not* ". . .using vincristine doses. . .").

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1033 Management of Advanced, Resistant Hodgkin's Disease and Non-Hodgkin's Lymphoma: A Phase II Study.

1035 Phase II Trial of Epirubicin in Hepatoma.

1037 Phase II Trial of Bisantrene in Patients With Metastatic Melanoma.

1039 Phase II Trial of Baker's Antifol in Patients With Recurrent or Inoperable Head and Neck Cancer.

1041 Phase II Trial of Mitoguazone in Children With Leukemia and Lymphoma: A Pediatric Oncology Group Study.

1043 Phase II Evaluation of Mitoxantrone in Advanced Carcinoma of the Stomach: A Southeastern Cancer Study Group Trial.

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